Livestock Nematode Infection in a Changing World: Investigating the European Situation

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Summary

Helminth infections are amongst the most common diseases in sheep and cattle. They are not only detrimental to the infected animals but also to the human populations depending on them for subsistence and income. Consequently, helminth infections are considered one of the major causes of reduced production and associated financial losses in livestock industry. Although helminth control is usually successfully achieved through anthelmintics drugs, the rapid spread of drug resistance in helminth populations highlights the need for alternative control strategies. In addition, global warming is expected to modify the population dynamics of many helminth species by influencing their development and survival outside the host thus modifying the spatial and seasonal epidemiology of helminth infection.

In this context, an EU-funded project, GLOWORM, has been conducted by several research groups in order to predict future changes in helminth infection in European livestock husbandry and develop suitable mitigation strategies. My thesis inscribes itself in the frame of this project and contributes to its objective first by providing new knowledge on current situation regarding helminth infection and management in European cattle and sheep with a focus on the financial consequences and second by exploring the relationship between weather parameters and population dynamic of nematodes in a temperate-alpine climate.

In the first chapter, I conduct a systematic review and meta-analysis of 88 studies investigating the reduction in performance in sheep due to nematode infection. Altogether the results show that infected sheep have 85%, 90% and 78% of the performance in uninfected individuals for weight gain, wool production and milk yield respectively. In addition the results allow a quantification of the relationship between faecal egg count and reduction in weight gain in lambs.

In the second chapter, using best-evidence synthesis, stochastic modelling and livestock production figures, I estimate the total annual losses of nematode infection in European dairy cattle and meat sheep industry to €902 and €372 million respectively. In addition, a review of the available literature indicates that anthelmintic are used in 38.4% and 91.5% of cattle and sheep farms respectively. Collaborations with other research groups are also presented here and provide new insight on the spatial distribution of nematodes and trematode infection as well as on anthelmintic resistance in Europe.

Finally, the third chapter investigates the development of *Haemonchus contortus*, one of the more relevant sheep nematodes, on alpine pastures in relation with meteorological variables using a generalized additive mixed modelling approach. The results indicate a non-monotonic relationship between the cumulated temperature in degree-days and larval development and a decisive influence of the weather conditions (temperature, rainfall and humidity) during the first week after the deposition of eggs on pasture.

Altogether the present thesis increases the current knowledge on several aspects of helminth disease in European livestock but also identifies areas were further research is warranted. The results will serve as a basis for predicting future changes in helminth infection epidemiology, setting priorities in resource allocation, developing sustainable alternatives to anthelmintics and will contribute to the efficient adaptation of helminth management in the face of the arising challenges in sheep and cattle industry.

Zusammenfassung

Helmintheninfektionen sind eine der häufigsten Erkrankungen bei Schafen und Rindern. Sie sind nicht nur schädlich für die Gesundheit der betroffenen Tiere, sondern auch für Menschen, die von diesen Tieren für ihren Eigenbedarf und Einkommen abhängig sind. Infolgedessen werden Helmintheninfektionen als eine der Hauptursachen von reduzierter Leistung und assoziierten finanziellen Verlusten in der Tierproduktion betrachtet. Obwohl sich Helmintheninfektionen durch Antiparasitika erfolgreich behandeln lassen, führt die schnelle Verbreitung von Resistenzen in den Helminthenpopulationen zu einem Bedarf an alternativen Parasitenbehandlungsmethoden. Zusätzlich wird davon ausgegangen, dass der Klimawandel die Populationsdynamik von mehreren Helminthenspezies beinflusst, indem sich ihre Entwicklung und Überleben ausserhalb des Wirts ändern. Als Folge davon wird sich auch die räumliche und saisonale Epidemiologie von Helmintheninfektionen verändern.

In diesem Kontext wurde von mehreren Forschungsgruppen ein EU-finanziertes Projekt (GLOWORM) durchgeführt, um zukünftige Änderungen der Helmintheninfektionen in der europäischen Kuh- und Schafzucht vorherzusagen und geeignete Mitigationsstrategien zu entwickeln. Meine Arbeit ist Teil dieses Projekts und leistet einen Beitrag zu diesen Zielen. Zunächst liefert sie neue Kenntnisse über die aktuelle Situation der Helmintheninfektionen und des Managements in europäischen Beständen mit einem Fokus auf die finanziellen Folgen von Helmintheninfektionen. Weiterhin wird die Beziehung zwischen verschiedenen Wettervariablen und der Populationsdynamik von Nematoden in einem gemässigten Gebirgsklima untersucht.

Im ersten Kapitel findet sich eine systematische Übersichtsarbeit und eine Meta-Analyse von 88 Studien, die die Leistungsreduktion in mit Nematoden infizierten Schafen untersuchen. Insgesamt zeigen infizierte Schafe 85%, 90%, und 78% der Leistung von nicht infizierten Schafen bezüglich Gewichtszunahme, Wollproduktion und Milchleistung. Ausserdem erlauben die Ergebnisse eine quantitative Schätzung des Zusammenhangs zwischen Eizahl im Kot und Reduktion der Gewichtzunahme der Lämmer.

Im zweiten Kapitel schätze ich anhand von Daten aus der Nutztierproduktion, mithilfe eines stochastischen Modells, in einem „best-evidence synthesis“ Ansatz, die gesamten jährlichen durch Nematoden verursachten Verluste in der europäischen Milchkuh- und Schlachtlämmerproduktion auf €902 und €372 Millionen. Anhand einer Literaturrecherche wird deutlich, dass Anthelmintika in 38.4% und 91.5% der Rinder- und Schafbestände benutzt werden. Ergebnisse der Zusammenarbeit mit anderen Forschungsgruppen werden hier präsentiert und liefern neue Daten über die räumliche Verteilung von Nematoden und Trematoden sowie die Anthelmintikaresistenz in Europa.

Im abschliessenden dritten Kapitel untersuche ich die Entwicklung von Haemonchus contortus, einem der wichtigsten Schafnematoden auf Alpenweiden in Zusammenhang mit Wettervariablen und mithilfe eines generalisierten additiven gemischten Models. Die Ergebnisse weisen auf einen nicht-linearen, nicht-monotonen Zusammenhang zwischen kumulierter Temperatur in Grad-Tagen und Larvenentwicklung hin. Weiterhin wurde ein starker Einfluss der Wetterbedingungen (Temperatur, Regenfall, Feuchtigkeit) während der ersten Woche nach der Ablage der Eier auf die Weide deutlich.

Zusammenfassend verbessert diese Arbeit das aktuelle Wissen zu verschiedenen Aspekten von Helmintheninfektionen in europäischen Nutztieren. Gleichzeitig weist sie auch auf Gebiete, die weitere Forschung brauchen, hin. Die Ergebnisse werden als Basis dienen, um zukünftige Änderungen in der Epidemiologie der Helmintheninfektionen vorherzusagen, Prioritäten in der Ressourcenverteilung zu setzen und nachhaltige Alternativen für Anthelmintika zu entwickeln. Damit werden sie beitragen zur effizienten Anpassung des heutigen Helminthenmanagements hinsichtlich der kommenden Herausforderungen für die Rind- und Schafproduktion.

INTRODUCTION

On livestock helminths

The term helminths describe diverse endoparasitic worms infecting animals in the whole world. They can be classified into three major groups: cestodes, trematodes and nematodes. Here is a short description of those three groups along with some examples of helminth species; this description is by far not exhaustive but rather focused on helminth species relevant to livestock husbandry:

1) Cestodes are segmented flatworms which dwell as adult worms in the digestive tract of their definitive hosts but also infect intermediate hosts during their pre-adult life stages. Although cattle and sheep are definitive hosts to some cestode species such as *Moniezia* spp., many cestodes use cattle and sheep as intermediate host: ingested larvae migrate outside the intestine and form cysts in different places within the host body. The cycle of the parasite is then completed when the intermediate host and the cysts are consumed by a potential definitive host. Particularly relevant in livestock industry are cestodes for which human is a definitive or accidental host because of their zoonotic potential (e.g. *Taenia* spp., *Echinococcu*s spp.).
2) Trematodes form the group of non-segmented flatworms, whose most relevant representatives are liver flukes (e.g. *Fasciola hepatica, Dicrocoelium dendriticum*). Liver flukes are characterized by an external life cycle involving snails and their ability to migrate outside the digestive tract of their host and establish themselves in the liver. Other relevant trematodes for domestic ruminants are *Calicophoron* spp. which infects the rumen.
3) Nematodes are round worms and by far the group with the largest amount of species considered detrimental to livestock health and productivity. The majority of livestock nematodes typically have an external cycle without intermediate hosts and rely on their natural resistance to survive in the environmental condition long enough to be ingested by a new definitive host. Livestock nematodes mostly infect the digestive tract, whereas some species (e.g. *Dictyocaulus* spp.) migrate to the lungs.

The effects of helminth infection on animals range from subclinical symptoms to death. In general, helminths are detrimental to the host's physiology either by depriving the host of nutritional elements or directly feeding on the host's tissue or blood, thus causing nutritional imbalance, deficiency or anaemia. Additionally, helminths can provoke tissue lesions and induce inflammatory reactions; the resulting pathologies and symptoms depend of the organ affected by the helminth infection (e.g. digestive tract, liver, lungs).

Parasitic helminths are ubiquitous pathogens and infect virtually all mammals species world [1, 2]. In livestock industry, which amounts worldwide to 40% of the total agricultural gross production [3], helminths infection is not only an issue regarding the health and welfare of animals but also impacts the human population depending on them for income and subsistence. In sheep and cattle, with a worldwide population of 1.2 and 1.5 billion heads ([4]), helminths infection is considered one of the major disease and first sources of production losses in both industrialized and developing countries [5–7]

Importance of the environment in livestock helminth ecology

A large part of all gastrointestinal helminths spend a part of their life cycle outside the definitive host: eggs are excreted with the faeces of the host and hatch in the environment; the larvae leave the faeces and either infect an intermediate host (e.g. liver flukes) or survive in the environment (e.g. most gastro-intestinal nematodes) before being ingested by another potential definitive host, completing the cycle. Thus environmental conditions during the external part of the cycle are determinant for helminth to thrive. Such conditions encompass abiotic factors (e.g. temperature, rainfall, soil pH, UV-exposure temperature, humidity) and biotic factors (e.g. intermediate host availability, presence of dead-end hosts, predation of eggs and larvae by insects and earthworms) [8]. In particular, climatic conditions are decisive for the geographic and seasonal occurrence of different helminth species. For example, the blood-sucking sheep nematode *Haemonchus contortus* develops and survives particularly well in warm and humid climates such as equatorial or subtropical regions whereas other nematodes such as *Teladorsagia circumcincta* are more adapted to cold climate [9].

Helminth control in livestock from past to present

Human population around the world have since long recognized the importance keeping their animal healthy in order to increase or secure their subsistence and profit. Records of medication applied to livestock can be traced back as far as the 2nd millennium BCE in Egypt and India [10, 11]. Concerning helminthic diseases, earliest mentions of anthelmintic treatment applied to animals date from the 13th century CE [12]. However, it is during the 20th century that there was a revolution in the chemotherapeutic worm control with the development of anthelmintic drugs. Anthelmintic drugs can be categorized in major classes referring to their molecular structure which will determine their mode of action and the species of helminths against which they are effective. Major anthelmintic classes are benzimidazole (broad spectrum, effective against nematodes and trematodes), levamisole (effective against nematodes and in higher doses against trematode), salicylanilides (effective against trematodes), praziquantel (effective against cestodes) and ivermectin (effective against nematodes).

A changing world

However, two important challenges are now arising in livestock production and have the potential to radically impact animal husbandry and health under the current management methods. First is the increasing resistance of many nematode and trematode species to anthelmintic drugs. Similarly to the way bacteria developed resistance to antibiotics, helminth populations around the world have quickly adapted to the intensive use of anthelmintics in the livestock sector. Resistance to widely used drugs such as benzimidazole have been reported worldwide and have already become an issue in several countries especially in the sheep industry [13, 14]. To make matters worse, although a large number of new anthelmintics drugs have been developed during the 20^{h} century, the development rate of new compounds has dramatically decreased since the 1980's and the conception of ivermectins. Since then, only two new classes of anthelmintics have been developed, namely amino-acetonitrile derivatives (eg. Monepantel, [15]) and octadepsipeptides (eg. Emodepside, [16]). Thus with resistances reported against almost every class of anthelmintics, including in one of the two only new anthelmintic classes developed in the last 30 years [13, 17], a future livestock industry without anthelmintics seems increasingly likely. Although attempts have been made to reduce the use of anthelmintic drugs and to develop alternatives to chemical worm control such as pasture rotation, mixed-species grazing, vaccination or breeding of resilient livestock [18, 19], those strategies require a better knowledge of helminth ecology, population dynamic and their impact on livestock in order to reach a level of efficiency compatible with modern livestock industry.

In addition to anthelmintic resistance, climate change due to global warming is a further arising challenge for livestock husbandry [20]: first through its direct effect on animals (e.g. heat stress) or through the modification of their environment and of the available resources (e.g. increased mortality due to natural disasters, changes in availability of water or grass) but also through the modification of host-pathogen interactions. In particular, helminth parasites which have a free-living stage or depend on the availability of an intermediate host to complete their life-cycle will be subject to climate-driven changes [21]. Not only will the geographical spread of helminths be modified, but also the seasonal pattern of occurrence will be altered [22]. Such changes have been already reported [23–25], and their incidence is expected to increase as global warming continues [26].

The GLOWORM project and the present thesis

Thus, anthelmintic resistance and global warming are both major game-changers which will modify livestock helminthic disease epidemiology and have important economic repercussions. In order to develop suitable mitigation strategies, we need first to better understand the coming changes and their consequences on modern livestock husbandry. In this optic, a consortium of several research groups across Europe including the Section for Veterinary Epidemiology and the Institute of Parasitology in Zürich started an EU-financed project called GLOWORM (http://www.gloworm.eu), which aims at answering those needs. The main objectives of GLOWORM are to improve diagnostic methods, to assess the current situation regarding trematode and nematode infection in European livestock and develop predictive models allowing the exploration of different climatic or resistance scenarios and finally to propose adapted strategies in order to mitigate the economic impact of helminth infection in the short and long-term.

The present thesis inscribes itself within the GLOWORM project and contributes to several of its objectives either through original research conducted in Zürich or through collaborations with the other research groups involved in the project. Although GLOWORM encompasses nematode and trematode infections, our work focuses mostly on nematodes and has three major objectives which are addressed in the three chapters of the thesis:

I. Improve the understanding of how nematodes impact on livestock production

The effect of helminth infection on different performance traits on livestock production has been extensively researched in parallel with the development of anthelmintic drugs. However, the reported results show a large variability and are sometimes contradictory. A preliminary inquiry showed that efforts have been made to summarize and synthesize the current knowledge for cattle, but less so for sheep. In particular: Dargie et al., [5] reviewed the pathomechanisms and economical implication of both nematode and trematode infection; Sanchez et al. [27] conducted a systematic review of the effect of anthelmintics administration on milk yield in cows and Forbes et al. [28], summarized the quantitative relationship between milk antibody level against *Ostertagia ostertagi* in milk production in cows.

In this first chapter, I intend to fill the gap in knowledge concerning sheep production. I use the rigorous methodology of systematic review and meta-analysis in order to obtain a reliable estimate of the effect of gastro-intestinal nematode infection on the three main production aspects of sheep husbandry: meat, milk and wool. In addition I use generalized linear modelling to evaluate the quantitative relationship between loss in production and infection intensity as indicated by faecal egg count. The results will help to comprehend the importance of nematode infection for sheep production and establish a basis for evaluating the financial losses in the second part of the thesis. The results of this systematic review have been published in the journal “Parasites and Vectors” [29].

II. Assessing the current European situation regarding helminth infection in livestock

Knowledge of the impact of nematodes in the livestock industry is an essential step toward better understanding the consequences of the predicted changes in helminth distribution and abundance due to global warming and rising anthelmintic resistance. In addition, the effect of nematode infection needs to be contextualized as a part of the total cost and benefits in livestock production. Only then can decision-makers and stakeholders set priorities and implement adapted mitigation strategies.

Although numerous European studies report prevalence and levels of infection in livestock at regional and national levels, the large variability in the methodology used to conduct the investigation and in the data coverage across the different countries makes a synthesis on a continental scale difficult. A further challenge is to connect the level of infection estimated in an animal population to financial losses: to our knowledge, only two studies delivered such estimates: one in the United-Kingdom for sheep [30] and the other for dairy cattle in Belgium [31].

In this chapter, I review the available European scientific literature of the past 15 years in order to identify suitable studies and using best evidence data and stochastic modelling, I evaluate the infection levels across Europe in dairy cattle and meat sheep. Then, using the relationship between infection level and reduction in performance as reported by Forbes et al. [28] for dairy cattle and computed in the first chapter of this thesis for sheep [29], and combining them with data on production and prices, I produce estimates and uncertainty intervals for the financial losses associated with nematode infection. Finally, in order to gain insight on the costs of helminth infection control, I also review the situation concerning deworming practices in Europe. The results will be submitted for publication in the journal “Veterinary Parasitology” [32].

In addition to estimating the nematode infection levels and associated losses in European livestock, a part of my work consisted of collaborations with several research partners in GLOWORM on other projects addressing the situation of helminth infection in European livestock: first, on a review on anthelmintic resistance conducted by Dr H. Rose from the University of Bristol and second, on a two-years coprological survey carried out in Ireland, Italy and Switzerland lead by Prof L. Rinaldi from the University of Napoli. The three resulting publications are included in the appendix at the end of this thesis [32–34].

III. Evaluating the influence of meteorological factors on nematode ecology

In chapters 1 and 2, I evaluated how nematodes already impact livestock production and economy. Here I explore how climatic and meteorological factors affect the ecology of nematodes. The motivation for this is twofold. First, together with the work on the current situation in Europe presented in the second chapter of this thesis, it will provide a basis for modelling future change in seasonal and geographical nematode occurrence and abundance in relation to climate warming as well as the associated impact on animal production. Second a better understanding of the population dynamic of parasite free-living stages will help to develop and implement more efficient alternatives to anthelmintic treatment such as pasture rotation or evasive grazing management [18].

The results presented here were obtained through field experiments during three consecutive years on the development of *H. contortus* on alpine pastures, This parasite is of particular interest since it is associated with substantial losses in sheep industry [19, 35] and prone to develop resistance to anthelmintics [36]. Moreover, although primarily associated with tropical and subtropical climate [9], it is reportedly expanding its range in northern Europe [24, 26]. Furthermore, resistance of the parasite to anthelmintic drugs has been reported in various European countries [34], further emphasizing the need for sustainable control solutions. Although several studies described the effect of temperature, humidity and rainfall on the development of the parasite, they mostly focused on only one of those parameters or considered them separately in their analysis, here I use generalized additive mixed models which allow to consider all those variables together and describe non-linear and non-monotonic relationships between meteorological predictors and the development of the parasite. In addition, previous studies were mostly conducted in warm tropical, sub-tropical, arid or semi-arid environments and there is little data on the development of *H. contortus* in pastures under more temperate climatic condition. The results presented in this chapter are planned to be submitted for publication in the journal “Ecological Modelling”.

<u>Contribution of the thesis defendant to the work presented here</u>

Chapter I: I conducted the literature review, performed the analysis and drafted the manuscript.

Chapter II: I reviewed and retrieved relevant information for the analysis, developed the model and drafted the manuscript.

Chapter III: I organized and conducted the seven first experimental trials during the years 2012 and 2013. I helped supervise Jasmin Steiner which conducted two additional trials during summer 2014 as part of her master thesis. I conducted the statistical analysis and drafted the manuscript.

Appendix (collaborations with other research groups):

- Review on anthelmintic resistance: I reviewed and retrieved the relevant scientific literature for Switzerland.
- Joint coprological survey in sheep flocks from Ireland, Italy, and Switzerland: Together with Dr Hertzberg I organized the sampling in Switzerland, collected the relevant information and managed the information on the sampled flocks in a database.

All my work has been done under the supervision of Prof P. Torgerson and Dr H. Hertzberg.

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CHAPTER I

Effect of gastro-intestinal nematode infection on sheep performance: a systematic review and meta-analysis

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ABSTRACT

BACKGROUND: Gastrointestinal nematode (GIN) infections are common in domestic sheep and impact directly and indirectly on the health of infected animals as well as on the associated economic production. In this study, we aim at summarizing the current knowledge on the influence of GIN infections on sheep production by conducting a systematic review. A subsequent meta-analysis of relevant studies was performed to provide an estimate of the effect of GIN infections on weight gain, wool production and milk yield.

METHODS: A literature search was performed on the CAB, Pubmed and Web of Science database for the period 1960-2012. Inclusion criteria were: 1) Measurement of at least one production parameter. 2) Comparison between groups of sheep with different nematode burdens. 3) Same conditions regarding all aspects except parasite burden between groups. 4) Quantitative measurements of one or more production traits.

RESULTS: Altogether, 88 studies describing 218 trials were included in this review. The majority of studies (86%) reported that GIN infections had a negative effect on production but this was reported to be statistically significant in only 43% of the studies. Meta-analysis indicated that performances of sheep infected with nematodes was 85%, 90% and 78% of the performance in uninfected individuals for weight gain, wool production and milk yield respectively. Our results suggest a possible reporting bias or small study effect for the estimation of the impact of GIN infections on weight gain. Finally, a general linear model provided an estimate for the decrease in weight gain in relation to the increase in faecal egg count of nematodes.

CONCLUSION: This study underlines the importance of GIN infections for sheep production and highlights the need to improve parasite management in sheep, in particular in face of challenges such as anthelmintic resistance.

Keywords: sheep, gastro-intestinal nematodes, impact, weight, wool, milk, production

BACKGROUND

Gastro-intestinal parasitism is one of the most common infections in livestock. Clinical signs and sequelae are dependent on the parasite fauna present and the intensity of infection. In sheep, these can range from subclinical weight loss to lethal pathologies such as anaemia, diarrhoea and severe protein loss [1]. In addition, parasitism can have indirect consequences on metabolism such as mobilisation of proteins for an immune-response, reduced feed intake due to anorexia or increased susceptibility to other pathogens [2],[3],[4]. Since the 1960s the use of anthelmintics has become an important strategy to control nematode infections in livestock and increase their production performance [5]. For example, Sanchez *et al.* [6]reported the results of a meta-analysis which concluded that dairy cattle gained an estimated increase in milk production of 0.35 kg/day following treatment against gastro-intestinal nematodes.

According to the Food and Agriculture Organisation [7] the sheep population amounted to 1.2 billion in 2012, distributed as follow: Asia, 44.9%, Africa, 27.6%, Europe, 11.1 %, Oceania, 9.1% and Americas, 7.3%. Worldwide, sheep production for 2012 was 10 million tons of milk, 8 million tons of meat and 2 million tons of wool. Distribution of meat production is correlated with distribution of sheep population whereas milk production is mainly based in the Mediterranean region and the Near East and wool production is proportionally more important in Oceania and Asia [7],[8].

Sheep represent an important, source of income in many countries [8],[9] and although the effects of parasitism on production have been recognized [10], there is still a need to quantify these losses. Anthelmintic resistance and climate change is likely to alter the geographical distribution of parasites and their impact on production animals, thus increasing the need for a clear understanding of the cost of parasitism in order to develop sustainable control strategies. [10],[11].

Systematic reviews and meta-analysis have been widely used to summarize results of different studies made on one particular subject. The increased sample size obtained when combining studies as well as the possibility to identify error sources such as publication bias improve the quality of the analysis and strengthen its conclusions. In particular, in medical research, those methods are frequently used to measure the efficacy of a treatment or assess the relationship between risk factors and a medical condition [12].

Here we undertake a systematic review to identify studies which evaluated the impact of gastrointestinal nematodes on different aspects of sheep production and summarize their results. Meta-analysis was then applied to the data in suitable studies to evaluate the effect of gastrointestinal nematode (GIN) infections in sheep on weight gain, wool production and milk yield which are the main economic purposes of sheep breeding [9],[13]. Finally, since effects of parasitism are expected to depend on the parasite burden [10], we also analysed the relation between quantitative egg excretion (used as a proxy for parasite burden in young animals [14]) and production performance.

METHODS

The methodology followed the "Preferred Reporting Items for Systematic Reviews and Meta-Analyses" (PRISMA, [15]) recommendations for improving the standards of meta-analyses. A PRISMA check list is provided as supplementary material to this publication (see Additional file 1). Statistical analysis and figures were made using the R statistical program [16].

Search strategy

The databases CAB, Pubmed and Web of Science were searched for the period 1960-2012 in order to retrieve relevant studies. Three production traits were taken in consideration: weight gain, milk yield and wool production. Searches were performed using different key words distributed among three search terms: [nematode / parasite / anthelmintic / parasite control] AND [weight / growth / wool / fleece /milk / production] AND [sheep]. All possible combinations of the three terms were used (e.g. anthelmintic AND fleece AND sheep).

Inclusion and exclusion criteria

Studies were first screened by scanning the title and abstract. Suitable studies were retained for more detailed examination. Studies were then selected for inclusion if they met the following criteria:

(1) A production parameter was measured (weight gain in lambs, wool production or milk yield).

(2) There were at least two groups of sheep which differed in their gastro-intestinal nematode burden (e.g infected sheep group vs control or dewormed group vs control).

(3) There were no other reported differences between the groups (e.g. feeding, breed, housing, age, infection with trematodes).

(4) The report quantified the production of each group or whether there was a significant difference between groups.

For studies describing more than one trial, each trial was included separately in the review. Additionally, for studies where more than one group were compared to the control group, each group being compared with the control group was considered as a separate trial. Finally, for studies measuring more than one production trait, the recorded gain in each production trait was considered as a separate trial.

Trials were classified into two categories:

a) Infection/control trials: trials with an infected group (INF) and a control group (CONT) with no or a negligible nematode infection (animals raised and kept in a nematode-free environment or regularly treated and with a mean faecal egg count (FEC)<50 eggs per gram (EPG) determined by repeated measurements over the trial's duration).

b) Burden trial: Trials which compared production between two groups of nematode infected sheep but in which one group had a high parasite burden (HPAR) and the other group had a lower burden (LPAR).

Subsequently, only trials of the type infection/control were included in a meta-analysis of the effect of infection status on performance. In addition an analysis on the effect of nematode burden on performance was undertaken using all trials (infection/control and burden types) for which FEC was monitored in every group (based on repeated measurements over the duration of the trial).

Effect of infection status on performance

Using the meta and metafor packages in R [17],[18], a meta-analysis was undertaken to evaluate the effect of infection status on production. To construct a confidence interval around the final gain in production, only trials reporting a standard error of the measured outcome were included in the analysis.

A standardized measurement of the gain in production was obtained by computing the ratio of the performance in the INF group over the performance in the control group (no parasite burden). This allowed comparison between different studies, since the reported performance (in grams of body weight/fleece or litres milk) can be influenced by other factors such as breed, feeding or trial duration or was measured with different units between different studies (e.g wool production measured either in grams of wool at shearing or in mm wool growth).

Since this standardized measurement is a ratio, logarithmic transformation, as described in [19], was used for the computation of confidence intervals and to perform further analysis.

Analysis was performed separately for the three production traits (weight gain, wool production and milk yield) as well as for the type of nematode infection: either mixed species infection or mono-infection with *Haemonchus contortus*, *Trichostrongylus colubriformis* or *Teladorsagia (Ostertagia) circumcincta* . Additionally, only studies performed on growing animals less than one year old were included in analysis measuring weight gain.

Linear regression test for funnel plot asymmetry [20] was conducted to control for publication bias or small-study effect and the fill-and-trim method [21] was used to compute an adjusted estimate of the overall effect when needed.

Relation between egg excretion and performance

We built a generalized linear model (GLM) to estimate the impact on production in relation to the faecal nematode eggs output. The measured outcome was defined as the log-transformed ratio of production of the infected group over the control. In addition to the log-transformed difference in mean FEC between the groups five additional explanatory variables were included in the model: 1) the absolute value of the latitude at which the trial was conducted (ranging from 0 at the equator to 90 at each pole) which served as proxy for a possible effect of climate [22],[23]; 2) trial duration in weeks, since the impact of a pathogen might not only depend on infection intensity, but also on infection duration [24] or development of immunity by the host [2];3) age classes of the animals (1-6 months or 7-12 months) since effect of parasitism and host response can vary with the age of the lambs [25],[26];4) study design (infected vs control, treated vs untreated or other) was added as a predictive variable since infection pressure and its fluctuation over the trial duration might differ between the different type of trials. In addition, infection course and host response might differ between experimentally or naturally acquired parasite infection [27]; 5) FEC diagnostic method (flotation or centrifugation) was also included since it might influence the estimate of parasite burden in animals [28],[29]. Additionally, trials were assigned weight in the model according to their sample size. The model was constructed using backward selection based on the Aikake Informaton Criterion (AIC).

Similarly to the meta-analysis on the effect of infection status, we considered trials separately, depending on the three production traits measured as well as for the species of nematodes infecting the animals. However only trials measuring weight gain in lambs with mixed parasite infection were in sufficient quantity to provide a robust model (n=73) and thus, only those trials were used for modelling. Finally, we also investigated the relationship between FEC and nematode burden in studies which necropsied animals and performed a worm count of the whole gastrointestinal tract.

RESULTS

Searching the three databases, a total of 45402 results corresponding to 11873 studies were obtained. Of these, 265 studies remained after an initial screening of titles and abstract. Finally 85 studies were included following full paper review. The main reasons for excluding studies were: study on agent other than nematodes, study on species other than sheep, production parameters of interest not measured and difference between the experimental groups regarding aspects other than parasite burden (e.g. food, breed). During this process, three additional studies were identified from the cited references of screened studies and also included in the review resulting in a total of 88 studies [30–117].

These 88 studies described a total of 218 trials. Twenty-two studies described only one trial. The other 66 studies included at least two trials. Mean sample size in the trials was 49 (median: 20, range: 8-500) and average trial duration was 16 weeks. Gain in production was assessed by treating animals with anthelmintics in 42 studies, through experimental infection in 40 studies, through different pasture management methods (e.g. pasture rotation) in five studies and by comparing animals with naturally high and low FEC in one study. Studies originated from 23 different countries. The United-Kingdom and Australia were the countries with the most studies (18 and 12, respectively) and account for more than one third of the total studies included in this review (Table 1).

Table 2 shows a summary of the reported effect of parasitism on production in sheep. Altogether, 187 trials (85.8%) reported a negative effect of nematode infection on production, with 94 (43.1%) of them reporting a statistically significant effect. In contrast, a positive effect of parasitism on production was found in 24 trials (10.9%) and seven (3.2%) trials reported no differences in production between parasitised and control animals.

Altogether, statistical testing of the effect of parasitism on production was reported in 183/218 trials. There was no significant difference in the proportion of trials reporting a p-value between trials describing a negative effect of parasitism and those reporting a positive effect (159/187 vs 18/24, Fisher exact test: p=0.237).

However, a larger proportion of trials reported a significant negative effect of parasitism compared to trials reporting a significant positive effect (94/159 vs 2/18, Fisher exact test: $p<0.001$).

Effect of infection status on performance

A total of 94 trials were of the type infection/control and met requirements to be included in the meta-analysis (70 trials measuring weight gain, 5 trials measuring milk yield and 19 trials measuring wool production).

In 78/94 trials, a negative effect of parasitism on production was reported (weight gain: 59/70, milk yield: 5/5, wool production: 14/19). However, in 14 trials (weight gain: 10/70, wool production: 4/19) parasitism was associated with an increased performance. Finally, in two trials (one measuring weight gain and one measuring wool production), the authors reported there were no differences between infected and control animals.

Results of the meta-analysis are summarized in Figure 1 and Table 3. Test for funnel plot asymmetry indicated a possible bias for trials reporting weight gain ($p= 0.032$) but not for wool production ($p= 0.307$) and milk yield ($p=0.336$). Figure 2 shows the funnel plots for the three production traits.

Altogether, estimates for the production ratio of infected animals over control were:

- 0.77 (95% CI: 0.74-0.79) for weight gain or 0.85 (95% CI: 0.82-0.88) after adjustment for reporting bias,
- 0.90 (95% CI: 0.86-0.93) for wool production,
- 0.78 (95% CI: 0.73-0.84) for milk yield.

In 75 trials, mean FEC over trial duration were reported for the infected group and ranged from 100 to 12000 EPG (for details see table 3).

Relation between parasite excretion and performance

The best model (AIC: 27.532) included only increases in FEC as a predictor of the weight gain ratio between HPAR and LPAR groups (21.37% of deviance explained). Figure 3 shows the observed effect of parasitism recorded in the trials and the estimate of the model.

Altogether, by mixed species infection, an increase in FEC of 100, 1'000 and 10'000 EPG resulted in the HPAR lambs gaining 0.85, 0.71 and 0.6 times the weight of the LPAR lambs, respectively).

Finally, in 9 studies, lambs from either the HPAR groups or both HPAR and LPAR groups were necropsied and worm counts of the whole gastrointestinal tracts were performed. Altogether, worm count ranged from 30 to 41'718 and there was a positive correlation between mean FEC before slaughter and worm count (n=26, spearman's rho= 0.71, $p<0.001$).

DISCUSSION

In this systematic review, a number of studies describing the relation between parasite infection and production in sheep were identified. The large majority of studies focused on the effect of parasitism on weight gain and relatively few studies measured other parameters such as wool production or milk yield.

Altogether, the large majority of the trials reported a negative effect of parasitism on production only 58.3% of the trials for which a p-value was provided found this effect to be statistically significant. This lack of statistical significance could be due to the relatively small sample size in many of the studies as the median sample size in all the studies included in this review was only 20.

When looking at the trials comparing parasite-free and infected animals, the results of the meta-analysis indicate that, in parasite infected animals, the production in terms of weight gain, wool, and milk is respectively 77, 90 and 78% of the production of parasite-free animals. Analysing the separate impact of different species of nematodes gave similar estimates, with wool production being less influenced than weight gain by parasitism.

Testing for funnel plot asymmetry indicated that trials measuring weight gain were probably biased. Therefore the adjusted estimate of infected animals gaining 85% of the weight of non-infected animals seems more reliable than the 77% unadjusted estimate.

In contrast, no bias was detected following the meta-analyses of trials measuring wool production and milk yield. However, testing for bias is unreliable when the meta-analysis includes a small number of studies [118]. Thus there is the possibility of bias in the estimates of the effect of parasitism on wool production and milk yield presented in this review. If that is the case, it is likely that, similarly to weight gain, our analysis overestimates the true impact of parasitism on those production traits.

Nevertheless, our results indicate that milk yield and weight gain are much more influenced by parasitism than wool production. Coop *et al.* [2] proposed that sheep respond to parasitism by shifting resource allocation with higher priority to maintaining vital body function, with other function such as weight gain and lactation being given a lower priority, and thus more likely to receive less resources in case of parasitism. It is possible that wool growth is part of sheep vital functions, which might explain the smaller effect of parasitism on this parameter.

In a review of the effect of parasitism in dairy cow production, Sanchez *et al.* [6] noted that level of parasitic infection is likely to be an important factor determining the effect on the milk yield and probably accountable for the large variation of the effect reported in the different studies. Similarly, only a minority of the studies included in the present review reported a level of infection, either by describing the initial parasite dose in case of experimental infection trials or by sacrificing animals to perform a post-mortem worm count.

In another meta-analysis, Kipper *et al.* [119] estimated that parasite- infected pigs had a daily weight gain 31% inferior than non-infected individuals. Kipper did not discriminate between the different species of parasite when estimating their impact. He argued that the main effect of parasitism was due to the host adaptation to an infection and its immune response rather than to the species involved. The present study seems to support this argument since the estimate of the impact of the different nematode species considered separately were quite similar to the overall estimates for each production trait. However, because of the small number of trials for each separate nematode species, those estimates have to be interpreted with caution.

While FEC is usually considered a reliable indicator of nematode burden in small ruminants [14],[72],[120], some authors pointed out that the relationship between both variables might be more complex and involves other factors such as parasite density and diversity [121],[122] or host age and development of immunity [123],[124]. In this review, we found a strong relationship between FEC at slaughter and gastrointestinal worm count in lambs. It must be noted, though that those were averaged values which did not allow to account for individual variability and that the amount of groups for which worm count was reported was small (n=26).

In the GLM presented here, increase in FEC was the only variable included in the best model. It was significantly associated with a decrease in weight gain and explained 21 % of the total deviance. None of the other variables tested in the analysis were selected in the final model. However, because of a strong heterogeneity and a lack of precise information in the included studies, we summarized the variables study design and FEC diagnostic method into two or three rough categories (e.g. flotation *vs* centrifugation). This simplification might limit the ability of the model to detect an effect for those variables. For the same reason, other potentially relevant predictors such as breed, diet or co-infection with other pathogens could not be included in the analysis.

Although, alternative indicator such as plasma antibodies or pepsinogen level have been proposed [125], the results of this review corroborate that FEC can help evaluate nematode burden and its impact on weight gain in lambs. Additionally, procedures requiring blood sampling of individuals is more expensive in term of time and resources than FEC which make them less attractive for monitoring purpose. However, other less invasive parameters such as body condition or FAMACHA scores have proven themselves helpful in the frame of targeted selective treatments [126] and should be further propagated.

Finally, most of the studies identified with naturally infected animals used classical anthelmintic compounds in their experimental design. Although the efficacy of such products is widely acknowledged, increasing resistance of GIN to anthelmintics is reported worldwide [127],[128]. This review demonstrates that an increase in non-responsiveness to classical anthelmintic will have an important impact on sheep production and underlines the need for alternatives to chemical worm control such as pasture management, resistant breed or vaccination [129].

CONCLUSION

This study confirms the importance of GIN infections on sheep performance and underlines the advantages of parasite control in production animals. The consequences of GIN infections seem to be similar for different species of parasites but seem to influence milk yield and weight gain more than wool production.

COMPETING INTERESTS

The authors declare that they have no competing interests.

AUTHORS' CONTRIBUTION

PT and HH participated in the design of the study. FM performed the literature search and carried out the analysis under PT and HH guidance. FM drafted and finalized the manuscript and all authors contributed to and approved the final version.

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Chapter I - Tables and Figures

Table 1: Country of origin of 88 studies assessing impact of parasitism on production trait of sheep.

Europe (38)	***N***	**Oceania (21)**	***n***	**Americas (14)**	***n***	**Africa (10)**	***n***	**Asia (5)**	***N***
UK	18	Australia	12	Brazil	7	Kenya	5	India	1
Spain	5	New-Zealand	9	USA	4	Ethiopia	2	Indonesia	1
Greece	4			Argentina	1	South-Africa	2	Iraq	1
Italy	4			Mexico	1	Nigeria	1	Malaysia	1
France	3			Venezuela	1			Pakistan	1
Denmark	1								
Germany	1								
Ireland	1								
Switzerland	1								

Table 2: Effect of gastro-intestinal nematode infection on production in sheep reported in 218 trials.

Reported effect of parasitism on production	Measured production trait	Total number of trials	Number of trials reporting a p-value	Number of statistically significanttrials (p<0.05)	% of statistically significanttrials
Negative	weight gain	147	122	74	60.66
	wool growth	24	21	11	52.38
	milk yield	16	16	9	56.25
	total	**187**	**159**	**94**	**59.12**
Positive	weight gain	18	15	2	13.33
	wool growth	4	1	0	0.00
	milk yield	2	2	0	0.00
	total	**24**	**18**	**2**	**11.11**
None	weight gain	6	6	0	0.00
	wool growth	1	0	0	0.00
	milk yield	0	0	0	0.00
	Total	**7**	**6**	**0**	**0.00**

Table 3: Meta-analysis of 94 trials on the estimated effect of parasitic infection on sheep performance.

Production trait	Infection type	Number of trials	Ratio production infected/control	*95% C.I.*	Number of trials reporting egg excretion	Mean number of eggs per gram faeces in infected animals
weight gain	Mixed species[1]	30	0.74	0.71-0.77	22	2336
weight gain	*H. contortus*	20	0.79	0.71-0.87	20	4019
weight gain	*T. colubriformis*	12	0.78	0.71- 0.87	12	1070
weight gain	*T. circumcincta*	8	0.81	0.66-0.99	4	296
wool production	Mixed species[1]	14	0.9	0.86-0.93	11	3788
wool production	*H. contortus*	2	1.04	0.96-1.13	2	7585
wool production	*T. colubriformis*	2	1.02	0.95-1.1	2	1359
wool production	*T. circumcincta*	1	0.83	0.81-0.86	1	201
milk yield	Mixed species[1]	5	0.78	0.73-0.84	1	527

[1] Main species were of the genus Haemonchus, Teladorsagia, Trichostrongylus, Cooperia and Nematodirus

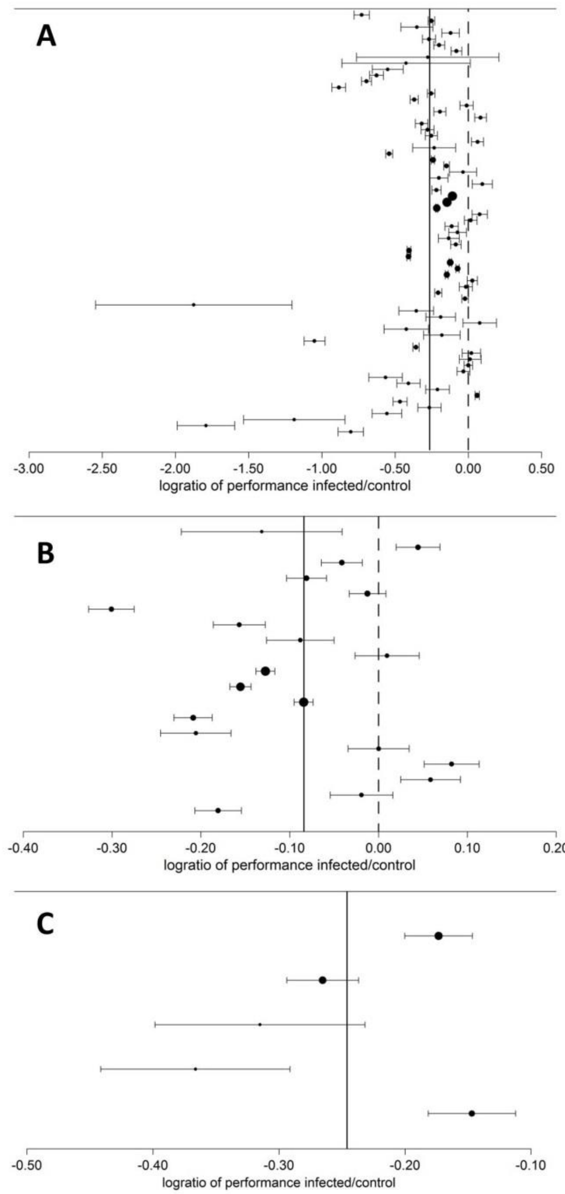

Figure 1: Forest plots of 94 trials included in the meta-analysis of impact of gastro-intestinal nematode infection on weight gain (A, n=70), wool production (B, n=19) and milk yield (C, n=5) in sheep. Black dots represent the log-transformed ratio of performance of the infected over the control group in each trial. Dot sizes are proportional to the sample sizes in the trial and horizontal bars give the standard error of the estimate. Vertical dotted lines indicate the zero (no effect of nematode infection on production) and vertical continuous lines show the overall estimate for all the trials in each performance trait.

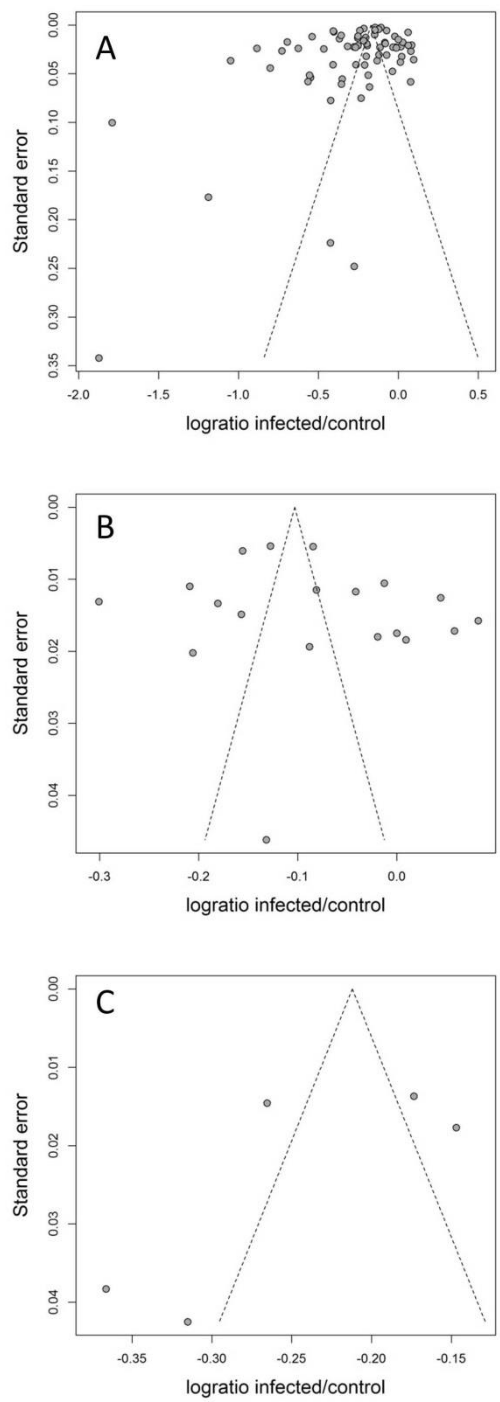

Figure 2: Funnel plots with 95% pseudo-confidence limits of 94 trials included in the meta-analysis of impact of nematodes on weight gain (A, n=70), wool production (B, n=19) and milk yield (C, n=5) in sheep. Treatment effect (log-transformed ratio of performance of infected over control animals) is given on the X-axis and standard error of the estimate is represented on the Y-axis.

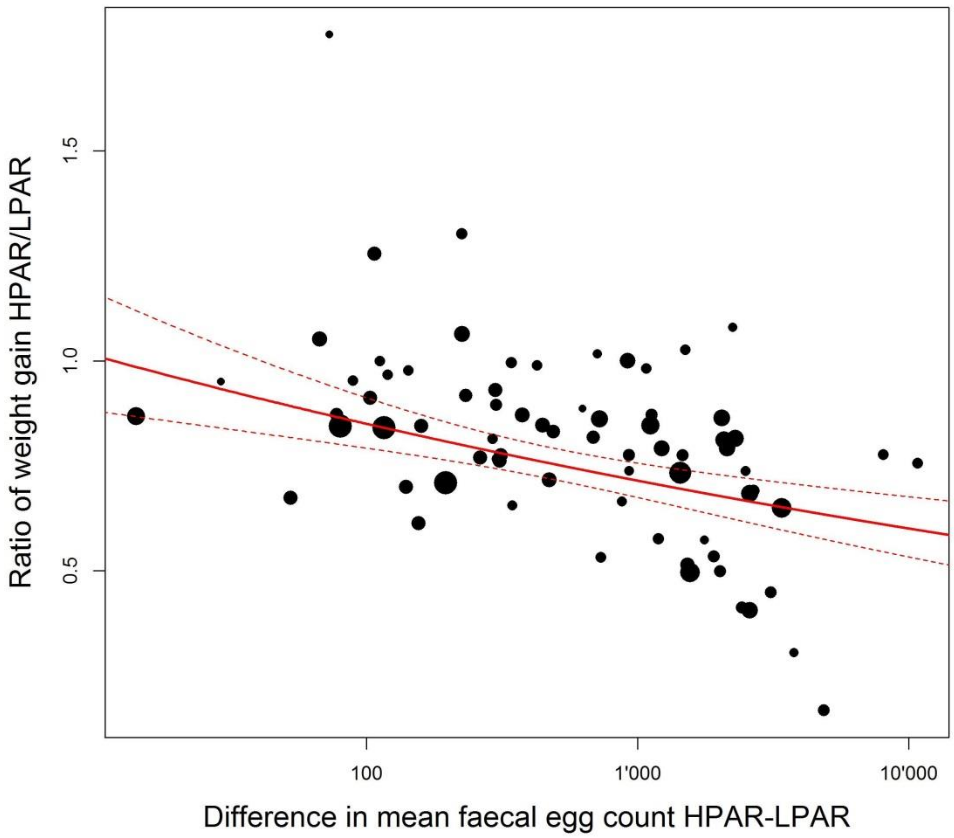

Figure 3: Decrease in weight gain of sheep by increasing infection level with mixed species of gastrointestinal nematodes. Mean difference in faecal egg counts between low parasite burden animals (LPAR) and high parasite burden animals (HPAR) is used as an indicator of level of infection and shown on the X-axis. Y-axis shows the ratio of weight gain of HPAR over LPAR. The continuous line shows the estimated effect of nematode infection with a 95% confidence interval (dotted lines) computed with a Generalized Linear Model using the results of 73 trials (black dots with sizes proportional to sample size of the trials).

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Chapter I - Annex A

PRISMA checklist

PRISMA 2009 Checklist

Section/topic	#	Checklist item	Reported on page #
TITLE			
Title	1	Identify the report as a systematic review, meta-analysis, or both.	23
ABSTRACT			
Structured summary	2	Provide a structured summary including, as applicable: background; objectives; data sources; study eligibility criteria, participants, and interventions; study appraisal and synthesis methods; results; limitations; conclusions and implications of key findings; systematic review registration number.	24
INTRODUCTION			
Rationale	3	Describe the rationale for the review in the context of what is already known.	25-26
Objectives	4	Provide an explicit statement of questions being addressed with reference to participants, interventions, comparisons, outcomes, and study design (PICOS).	26
METHODS			
Protocol and registration	5	Indicate if a review protocol exists, if and where it can be accessed (e.g., Web address), and, if available, provide registration information including registration number.	NA
Eligibility criteria	6	Specify study characteristics (e.g., PICOS, length of follow-up) and report characteristics (e.g., years considered, language, publication status) used as criteria for eligibility, giving rationale.	26-27
Information sources	7	Describe all information sources (e.g., databases with dates of coverage, contact with study authors to identify additional studies) in the search and date last searched.	26-27
Search	8	Present full electronic search strategy for at least one database, including any limits used, such that it could be repeated.	26-27
Study selection	9	State the process for selecting studies (i.e., screening, eligibility, included in systematic review, and, if applicable, included in the meta-analysis).	27
Data collection process	10	Describe method of data extraction from reports (e.g., piloted forms, independently, in duplicate) and any processes for obtaining and confirming data from investigators.	NA
Data items	11	List and define all variables for which data were sought (e.g., PICOS, funding sources) and any assumptions and simplifications made.	27
Risk of bias in individual studies	12	Describe methods used for assessing risk of bias of individual studies (including specification of whether this was done at the study or outcome level), and how this information is to be used in any data synthesis.	NA
Summary measures	13	State the principal summary measures (e.g., risk ratio, difference in means).	28
Synthesis of results	14	Describe the methods of handling data and combining results of studies, if done, including measures of consistency (e.g., I^2) for each meta-analysis.	28

PRISMA 2009 Checklist

Section/topic	#	Checklist item	Reported on page #
Risk of bias across studies	15	Specify any assessment of risk of bias that may affect the cumulative evidence (e.g., publication bias, selective reporting within studies).	28
Additional analyses	16	Describe methods of additional analyses (e.g., sensitivity or subgroup analyses, meta-regression), if done, indicating which were pre-specified.	29
RESULTS			
Study selection	17	Give numbers of studies screened, assessed for eligibility, and included in the review, with reasons for exclusions at each stage, ideally with a flow diagram.	30
Study characteristics	18	For each study, present characteristics for which data were extracted (e.g., study size, PICOS, follow-up period) and provide the citations.	30, table 1-2
Risk of bias within studies	19	Present data on risk of bias of each study and, if available, any outcome level assessment (see item 12).	NA
Results of individual studies	20	For all outcomes considered (benefits or harms), present, for each study: (a) simple summary data for each intervention group (b) effect estimates and confidence intervals, ideally with a forest plot.	31, table 2, fig. 1
Synthesis of results	21	Present results of each meta-analysis done, including confidence intervals and measures of consistency.	31, table 2
Risk of bias across studies	22	Present results of any assessment of risk of bias across studies (see Item 15).	31, fig. 2
Additional analysis	23	Give results of additional analyses, if done (e.g., sensitivity or subgroup analyses, meta-regression [see Item 16]).	32, fig. 3
DISCUSSION			
Summary of evidence	24	Summarize the main findings including the strength of evidence for each main outcome; consider their relevance to key groups (e.g., healthcare providers, users, and policy makers).	32
Limitations	25	Discuss limitations at study and outcome level (e.g., risk of bias), and at review-level (e.g., incomplete retrieval of identified research, reporting bias).	32-33
Conclusions	26	Provide a general interpretation of the results in the context of other evidence, and implications for future research.	34
FUNDING			
Funding	27	Describe sources of funding for the systematic review and other support (e.g., supply of data); role of funders for the systematic review.	35

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For more information, visit: **www.prisma-statement.org**.

Chapter I - Annex B

Supplementary material

Table 1: Qualitative review of the effect of gastro-intestinal nematode infection on sheep fertility and mortality

Author	Year	Country	Parameter	Method	Sample size	Results
Pandey	1984	Morocco	fertility	treated vs non-treated	313	Significantly higher fertility rate and lower abortion and still-birth rate and lamb mortality rate in treated ewes
Gatongi	1997	Kenya	fertility	treated vs non-treated	150	Significantly smaller age at first lambing and shorter lambing interval in treated ewes
Thomson	2000	Syria	fertility	treated vs non-treated	300	No effect of treatment on ewe productivity (no of lambs alive 85 days after exposure to ram)
Fernandez-Abella	2006	Uruguay	fertility	infected vs control	25	Significantly higher number of unruptured follicles and of corpora lutea in non-infected sheep. No effect on ovulatory efficiency detected.
Gaglio	2010	Italy	fertility	infected vs control	20	Significantly higher sperm concentration, motility and fertility in infected animals.
Lindahl	1963	United States	mortality	different managements	348	no significant differences in mortality between lambs on clean and contaminated pastures
Bekele	1988	Ethiopia	mortality	pathological survey	146	5% of necropsied sheep died due to either endo, ecto parasitism, foot and mout disease or local abscess
Gatongi	1997	Kenya	mortality	treated vs non-treated	150	No statistically significant effect of anthelmintic treatment on lamb mortality
Handayan	1998	Indonesia	mortality	treated vs non-treated	64	Significant effect of treatment on lamb mortality
Mukasa-Mugerwa	2000	Ethiopia	mortality	pathological survey	1413	9 and 13% of mortality caused by endoparasites in Horro and Menz lambs respectively
Kagira	2001	Kenya	mortality	pathological survey	366	20% of sheep presented for pathological examination died of gastro-intestinal helminthosis
Mandal	2007	India	mortality	pathological survey	4628	5% of necropsied sheep died due to endoparasitism
Tibo	2010	Ethiopia	mortality	treated vs non-treated	686	Significantly higher mortality in lambs in high frequency anthelmintic treatment group compared to lambs in low frequency treatment and control groups

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CHAPTER II

First assessment of production losses due to nematode infection in European dairy cattle and meat sheep

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ABSTRACT

Gastro-intestinal nematode infection is associated with reduced production in livestock animals, and thus with financial losses. In this study we aim at evaluating those losses in European dairy cattle and sheep meat industry. We retrieved available data on recognized indicators of nematode burden in dairy cows (antibody-level in bulk tank milk) and meat lambs (faecal egg count) for six main bio-economic regions across Europe. Using probability distribution and Monte-Carlo procedures we modelled nematode infection levels in those regions and estimated the financial losses in each country based on population and economic figures from the Food and Health Organisation and the European Union databases. In addition we evaluated the use of anthelmintics in Europe for dairy cattle and sheep.

Altogether there were different spatial patterns for nematode infection levels for cattle and sheep. For dairy cattle, annual production losses were estimated to €987 million in 30 countries (95% uncertainty interval: 818-1’241) which corresponds to approximatively 1.9% of the total production. In contrast, total losses were lower in meat sheep and amounted to €345 million in 33 countries (95% uncertainty interval: 157-477) but corresponded to a larger part of the total production (8.5%). In average, production losses amounted to €31 per dairy cow and year (range 0.5-92) and €7 per slaughter lamb (range 0.5-18).

Although our estimates only represent a part of the total costs of nematode infection in livestock industry and despite limitations due to the scarcity of data in certain regions, the results presented here appraise the impact of gastro-intestinal nematode infection on European livestock, evaluate the relevance of parasite control and will serve as a tool for planning of mitigation measures according to specific needs.

Keywords: livestock, Europe, gastro-intestinal nematodes, economic impact, Monte-Carlo simulations, modelling

BACKGROUND

In livestock, gastro-intestinal nematode (GIN) infection not only impacts the host physiology and health, but also indirectly affects human populations from associated production losses such as meat, milk or wool [1–3]. The economic implications of GIN infection are multiple and range from direct costs such as the prophylactic use of anthelmintics or treatment costs in animals with clinical symptoms to indirect losses following poor production performance in sub-clinically infected animals.

However, in order to be useful to decision-makers and stake holders, the effect of nematode infection needs to be expressed in costs and contextualized as a part of all the different variables influencing livestock production [3,4]. Additionally, predicted changes in helminth distribution and intensity of infection due to global warming and increase in anthelminthic resistance (Mas-Coma, 2008; Kaplan, 2012) stress out the need to understand the exact repercussions of nematode burden in order to implement adapted mitigation strategies.

In Europe, although numerous studies have been conducted in order to assess prevalence and intensity of nematode infection in several countries, only a few attempts have been made to produce estimates of the associated losses [1,5]. This is partly due to the heterogeneity in the type of monitoring data collected and the difficulty to efficiently link data on nematode infection and production losses. However, recently, in great part due to EU-funded projects (i.e. PARASOL and GLOWORM), efforts have been made by several research groups in order to a) propose standard markers for nematode infection level in livestock [6,7], b) conduct international studies with harmonized sampling and testing procedures [8,9] and c) synthetize the existing knowledge and quantify the effect of nematode infection on production [10,11].

The work presented here inscribes itself in the In the continuity of those efforts: we apply the principle of best-evidence synthesis combined to Monte-Carlo procedures in order to bring together data on GIN infection as well as production and economic figures from European countries to model losses in production due to GIN infection in dairy cattle and meat lambs throughout the continent.

METHODS

Software:

Data was managed with Microsoft® Excel 2010. Maps and figures were made with QGIS [12] and Microsoft® PowerPoint 2010 and the statistical program R [13] was used for computations and iterations.

Study area and definition of regions:

The study area encompassed all the countries which are members of the European Union or part of the Schengen agreement. In addition, Albania, Bosnia-Herzegovina, Former Yugoslav Republic of Macedonia, Montenegro and Serbia, were also included. Since, many countries had only scarce or no data on nematode infection in livestock, we grouped countries in larger regions and pooled together all the data retrieved within each region to model the level of GIN infection, similar to the method proposed by MacDonald et al. [14]. We determined regions as group of countries considered comparable in regard of two aspects: socio-economic and bio-climatic. Socio-economic characteristics were assumed to reflect farm and herd management as well as production methods whereas bio-climatic features were thought to be representative of the environmental conditions in which parasites and hosts live. Definition of those bio-economical regions was done based on the World Health Organization (WHO) classification for the estimation of burden of diseases [15], the Köppen-Geiger climate classification [16] and biogeographical regions defined by the European Environmental Agency [17]. Overall we defined eight regions representing three socio-economic groups (WHO 1, 2 and 3) and four bio-climatic groups (Arctic-Boreal, Atlantic, Continental and Mediterranean (Figure 1). Additional information on the classification method as well as a detailed list of the countries included in each region can be found as supplementary material to this study (annex A).

Variables selection for the model:

A list of all variables included in the model is shown in Table 1. For the estimation of GIN infection level in dairy cattle and meat lambs we took advantage of variables which are commonly reported in the scientific literature, are considered reliable indicators of nematode infection level and whose relationship with production losses has been established [2,10,11]: for dairy cattle, we chose the level of antibodies against *Ostertagia ostertagi* in milk samples determined by ELISA and expressed as Optical Density Ratio (ODR). For lambs, we used the faecal strongyle egg count expressed as eggs per gram of faeces (EPG).

In order to account for natural variation inherent to biological processes, those variables were modelled assuming they follow a specific distribution controlled by a mean and a measure of variability. ODR is considered to follow a Gaussian distribution [1,10], that can be defined by the mean ODR and a standard deviation. On the other hand EPG follows a negative binomial distribution whose shape is controlled by the mean EPG and an aggregation constant *k* [18,19]. The variability of ODR and EPG is thus defined respectively by the standard deviation of ODR (sdODR) and the aggregation constant *k* of EPG (*k*EPG). We further modelled those variability parameters by retrieving values of sdODR and *k*EPG from the scientific literature (either directly reported or mathematically approximated from variance or interquartile range) and fitting a gamma-distribution on them. Variability measure on herd level was used to produce estimate and uncertainty intervals for the GIN infection level in the different bio-economic regions. In contrast, estimates on production losses were computed at the animal level in each country, and thus we used animal level measures of variability for that part of our analysis. Details on the fitted distribution for sdODR and *k*EPG both at the herd and animal level are given in table 2.

<u>Literature search and inclusion criteria:</u>

A literature search was performed to gather available data in the different European countries on the different parameters included in the model. We searched the CAB, Pubmed and Web of Science databases as well as Google and Google Scholar search engines for relevant peer-reviewed articles, thesis, conference papers and breeder association websites in English, German and French language.

For data on GIN infection level, we retrieved survey type studies conducted in countries from our study area for the period 2000-2015. We included studies with random or convenient sampling reporting a mean ODR, and a mean EPG at sampling time or longitudinal studies performed on animals with naturally acquired nematode infections in commercial farms representative of the country's agricultural practises and which reported the mean values of repeated measurement over a time period. Since faecal egg count is considered to be less reliable in adult sheep than in lambs ([20]) and meat production is essentially based on slaughtered lambs, , we included studies reporting strongyle EPG values for lambs or mixed lamb-adult groups but excluded studies in which only adult sheep were sampled.

Among those studies, some additionally reported measures of variability either at the herd or the animal level and were used to parametrize the variables sdODR and *k*EPG. However, because of the scarcity of reported values for animal level sdODR and herd level *k*EPG, we searched for additional studies published earlier than 2000 or conducted outside the study area and included the reported values to our analyses assuming that variability in ODR and EPG was an intrinsic characteristic of GIN infection and less likely to be influenced by the geographical region or time period.

Additional information on production parameters, management practices and production prices were retrieved from the available literature, governmental statistics as well as the FAO and Eurostat databases for the years 2011-2012 [21,22] and in case of missing values for a country, the average values of all countries within the same bio-economic region were used. For Denmark and Spain, data from national databases were used [23,24].

<u>Model construction:</u>

Figure 2 summarizes the different steps we used to model the financial impact of GIN infection on European dairy cattle and meat sheep production.

GIN infection level: The retrieved values for the different parameter in each study were then used to produce estimates at the bio-economical region level using Monte-Carlo approach:

In each bio-economical region, we estimated the mean ODR and EPG based on 1'000 iterated populations: for each iteration, $\mathbf{n_1}+\mathbf{n_2}+\ldots\mathbf{n_i}$ random ODR and EPG values were drawn respectively from a Gaussian or a negative binomial distribution, with mean $\boldsymbol{\mu_1}$, $\boldsymbol{\mu_2}$, … $\boldsymbol{\mu_i}$ corresponding to the values reported in each study $\mathbf{S_1}$, $\mathbf{S_2}$, …$\mathbf{S_i}$ with sample size $\mathbf{n_1}$,$\mathbf{n_2}$,…$\mathbf{n_i}$ The herd-level variability parameter (sdODR or *k*EPG) for each iterated population was drawn from a gamma distribution as described in Table 2. With this method, the iterated population has an assortment of ODR or EPG reflecting the values reported in different studies and proportional to each studies sample size. Thus larger studies will weigh more in the estimation of the mean ODR or EPG of the iterated population. The mean ODR and EPG of 1'000 iterated populations were then used to construct 95% uncertainty intervals for those parameters which were then used in our model.

Production loss: Again, we used Monte-Carlo procedures to estimate the production loss in dairy cattle and slaughter lambs in each bio-economical region with mean loss in production and 95% uncertainty intervals computed from 10’000 iterated populations of 1’000 individuals each:

For dairy cattle: The populations were generated with ODR values drawn from a Gaussian distribution with mean ODR randomly chosen from the 95% uncertainty interval computed for each bio-region and animal-level sdODR drawn from a gamma distribution (Table 2). Loss in milk production for each animal was then computed using the relationship described by Forbes et al. [10]. Animals with an ODR value < 0.5 were considered as unaffected by nematode infection [25]. For each cow with an ODR value >0.5, loss in milk production was estimated according to the following equation:

Milk loss$_{daily}$ = 1.591 - 3.164 * ODR

Where the intercept (1.591) and slope (-3.164) are derived from Forbes et al. [10]. Finally, the annual loss for each cow was defined as

Milk loss$_{annual}$ = Milk loss$_{daily}$ * days in lactation per year

with the number of days in lactation per year randomly drawn from a uniform distribution with lower and upper bounds 250 and 300 days based on data on average lactation length and calving interval [26,27]. Finally, since the equation described by Forbes et al. [10], was done based mostly on Belgian cows [28] and disease impact is expected to vary depending on production performance [29], we applied a correction factor to take in account differences in milk production performance based on data from the FAO [21].

Similarly for meat sheep, populations of 1’000 lambs were iterated with strongyle EPG values drawn from a negative binomial distribution with a mean chosen randomly within the 95% uncertainty interval for EPG in each bio-economic region and animal level *k*EPG drawn from a gamma distribution (Table 2). Lambs with EPG values <40 were considered unaffected by nematode infection. For lambs with EPG>40, production loss was derived from Mavrot et al. [11] and was defined as the ratio of weight gain by infection over weight gain without infection:

weight gain$_{infected}$/weight gain$_{non\text{-}infected}$ = exp[0.185 - 0.076 * log (EPG)]

Thus, the result of this equation gives the proportion of weight gained by an infected lamb in comparison to the weight it would have gained without the GIN infection.

Finally, the production loss is given by the following equation:

Loss in carcass weight = (CW-BW) - ([CW– BW] * [weight gain$_{infected}$/weight gain$_{non-infected}$])

CW is the average carcass weight at slaughter. BW is the baseline carcass weight corresponding to the amount of carcass weight reached by the age of two months before switching mainly on solid food and the establishment of GIN infection [30–32] and represents the part of the total carcass weight which is not influenced by GIN infection. For each country CW was defined according to FAO statistics [21]. For the estimation of BW, we retrieved data on the average weight at two months of age in lambs for each bio-region [33–61]. BW was then computed assuming that carcass weight being around 40% of the live-weight [62,63].

We also applied a correction to take into account that a part of the lamb's weight gain is stored as subcutaneous fat which is trimmed at slaughter. The proportion of trimmed fat on lambs was determined using data on fatness conformation at slaughter following EUROP-standardized assessment and was included in our model as a variable following a Gaussian distribution with mean=0.07 and standard deviation=0.01 [64,65].

The annual total production losses in each country were then computed by combining the results of the Monte-Carlo simulations in each bio-economic region to the country level data on dairy cows population and number of lambs slaughtered for the years 2011 and 2012. In addition, for cattle those values were adjusted for the proportion of cattle being kept on pasture in each country [66–70]. Details on production parameters for each country can be found as supplementary material to this publication (Annex 2).

Financial losses: Finally, the estimated losses in kilograms of cow milk and lamb meat were combined to the producer prices in order to obtain estimates of the total annual financial losses due to GIN infection in the different European countries. In addition we also computed the losses as a proportion of the total production and as losses per dairy cow and per lamb slaughtered per year. Financial losses were computed in Euros..

RESULTS

GIN infection level:

Altogether, we retrieved 11 studies for cattle [8,10,28,71–78] and 26 studies for sheep [9,79–103] reporting results for 9'60 cow herds and 1'077 sheep flocks in 5/7 and 6/7 bio-economic regions respectively. No information could be retrieved for the bio-economical regions WHO2/Mediterranean (Albania, Cyprus, Montenegro) and WHO3/Continental (Hungary) for both cattle and sheep. For the region WHO3/Arctic-Boreal (Estonia, Latvia, Lithuania), only studies on nematode infection in sheep were retrieved. In addition, information on variability of ODR and EPG at herd and animal levels were retrieved from 24 of those studies and from seven additional studies [8,10,28,71–79,82,84–86,88,89,93,97,103–113](Table 2).

Table 3 shows the estimations of the GIN infection level for dairy cattle and meat sheep in the different bio-economical regions. For dairy cattle, the highest estimated value for ODR was produced for the WHO1/Atlantic region (95% Uncertainty Interval [UI]: 0.758-0.768) and the lowest ODR was in the WHO1/Mediterranean region (95% UI: 0.461-0.493). In contrast, the highest estimate for FEC in sheep was found in the WHO1/Mediterranean region (95% UI: 646-1174) and the lowest in the WHO1/Atlantic region (95% UI: 320-500).

Production losses:

In dairy cow, average annual losses in milk production per cow were estimated to range between 42 kg (Bosnia) and 333 kg (Denmark). For lambs, the average carcass weight loss per animal ranged from 0.3 kg (Slovakia) to 2.8 kg (Latvia). Across Europe, production losses amounted to 1.9% (95% UI: 1.6-2.4) and 8.5% (95% UI: 3.9 -10.9) of the total production for dairy cattle and meat sheep respectively (Figure 3).

Financial losses:

In the whole study area, annual financial losses due to GIN infection were estimated to €987 million (95% UI: 818-1'241) for dairy cattle and €345 million (95% UI: 157-447) for slaughter lambs. When considering only members of the European Union, the estimated losses were €838 million for dairy cattle and €334 million for meat sheep. At the animal level, the average estimated financial loss in dairy cattle was €31 per cow and per year (range: 0.5-92); this figure increased to €47 per cow (range: 12-114) when considering only grazed cattle. For sheep, the loss was estimated to €7 per slaughtered lamb (range: 0.5-18). In term of proportion, the loss in dairy cattle production was in average 1.9 percent of the total milk production (95% UI: 1.6-2.4). For sheep, losses amounted in average to 8.5 percent of total production (95% UI: 3.8-10.9). Figures 4 and 5 show the estimates of losses for both production systems in each European country as total losses in million Euros and as losses per animal in Euros. Detailed information on the estimated losses for each country included in the analysis can be found as supplementary material to this article (Annex 2).

DISCUSSION

In this study, we reviewed the situation concerning GIN infection levels in dairy cattle and slaughter lambs in Europe and developed a model in order to evaluate the related financial losses. To estimate the overall burden of GIN infection, we took advantage of standardized methods that are commonly used to monitor parasite infection in livestock (milk antibody level against *O. ostertagi* for cattle and faecal strongyle egg count for sheep).

In sheep, it has been suggested that faecal egg count might not always be accurately reflecting the worm burden [114], and other indicators such as the FAMACHA score have been proposed [7]. However, faecal egg counts are still considered a reliable tool for monitoring parasite infection in small ruminants [115,116] and remain the most widely used method. Thus infection level expressed as EPG is a value reported much more often than any other indicators in the literature.

In dairy cattle, many efforts have been made since the 80's in order to develop specific tools to detect IgG against *Ostertagia ostertagi* and estimate the relation between IgG level and production loss [2,10,117,118]. Although this indicator variable measures only the antibody response against one nematode species, it has been shown that it was a reliable indicator of infection level and negatively correlated to production in cows naturally infected with multiple nematode species [10,28,118]. In Western Europe, many studies based on IgG detection in bulk tank milk were conducted in the past 10 years. In contrast, there was a lack of data from Eastern Europe with only one rather small study (32 farms investigated) for the region WHO2/Continental and none for the regions WHO2/Mediterranean, WHO3/Arctic-Boreal and WHO3/Continental. However, it seems reasonable to assume that the efforts that have been recently made to use anti-Ostertagia antibody level as a standard indicator of nematode infection throughout Europe [71,8,119,75] will be carried on in the future which will allow improving the estimates presented here.

The results of the parameter estimation show that there is a geographical pattern in GIN infection level across Europe: north-western countries tended to have higher infection level in dairy cattle whereas southern and eastern Europe tended to have the highest EPG values in sheep. Those patterns might be due to variation in the climate and the predominant species of GIN infecting the animals but might also reflect cultural and economic differences in farm management, especially since those patterns tend to correlate with the importance of each production type in the different regions.

Annual losses for all the countries included in the analysis were evaluated to approximatively €987 million for dairy milk production and €345 million for sheep meat. Proportionally however, nematode infection had a higher impact on sheep meat production (8.5% of total production) compared to dairy cattle (1.9%). The inclusion of production parameters in our model in addition to nematode-related parameters allowed us to produce realistic country-specific estimates. Thus, the losses in each country will be proportional to the total production but will also be influenced by factors such as average milk production per cow or weight at slaughter as well as current prices in the country. For example, although the highest EPG values were estimated for the region WHO1/Mediterranean, lambs in this region are usually slaughtered at a young age and with a lower weight (average carcass weight below 15 kg). Thus, the losses in the countries of this region (Greece, Italy, Portugal, Spain) are proportionally lower than in other countries such as UK or Norway with a lower GIN infection level but where lambs are fattened on a longer period (average carcass weight above 20 kg).Nevertheless, it must be noted that, as in any model, our analysis has several limitations. First, there was a lack of data concerning GIN infection level for many countries. Although we could fill the gap using pooled data from countries within the same bio-economical region and the Monte-Carlo approach allowed us to produce uncertainty interval reflecting the sample size and the variation between the studies pooled together, there is still a need in many countries for a better assessment of the prevalence and infection intensity of GIN in livestock. Likewise, because of the scarcity and heterogeneity of the available data in the different European countries, we used an approximation of the numbers of days in lactation per year for dairy cattle and the weaning weight for lambs and did take into account other factors such as breed, age, lactation and growth curves, or co-infection with other pathogens, although it is likely that country specific estimates for those variables would also improve our model's accuracy.

Additionally, the relation between nematode burden and loss in production is more complex than those described by our equations: for example our model did not discriminate between possible variations in the spectrum of GIN species infecting livestock across Europe or the breed of cattle and sheep used in the different countries. However, Forbes et al., [10] noted that although more data might help to adapt their chart depending on the geographical zone, the relationship between anti-O. ostertagi level and milk production was constant across different countries and climatic regions. Likewise, the equation describing the relationship between faecal egg count and weight gain in lambs is based on a synthesis of 75 trials conducted across the world and for which no effect of the geographical location could be detected [11]. Finally, another limitation might be the use of point estimate of the average milk and meat prices from the FAO and European databases for the estimation of financial losses. The inclusion of factors such as product pricing according to its quality or price variations due to fluctuation in supply and changes in the market price of animal product is likely to increase our model's accuracy and should be further investigated in future analyses.

Synthesis of aggregated data has been widely used in human and veterinary medicine in order to estimate prevalence [120,121], evaluate transmission risk [122], assess the socio-economic impact of a disease [123,124] or develop predictive models [125]. However, the results obtained with this approach have to be considered with caution and in the light of the limitations inherent to the methodology. Despite these drawbacks, the model presented here represents a compromise between complexity and availability of the information in order to produce realistic estimates and provide a first appraisal of the European situation concerning GIN infection in livestock and its impact on production. Additionally, our analysis highlights areas where more research and empirical data collection are needed. Finally, although only few similar study have been conducted at the national level, they are consistent with our results: in 2012, Charlier et al. [1] estimated that the financial benefit in milk production by anthelmintic treatment in Belgian dairy cows amounted US$47 (€60) per lactation whereas our estimate of the annual losses in

Belgian dairy cattle lies around €62 per cow (€69 when considering only grazed cows). Concerning meat sheep: in 2005, Neuwhof et al. [5] evaluated the loss in weight gain due to nematode infection to cost GB£4.7 (€6.4) per slaughtered lamb and a total of GB£60 million (€100 million) to the whole British sheep industry every year. Again, those figures are close to our model's estimates (€8.2 per lamb, €112 million in total).

In the present study, we focused on the loss in production due to GIN infection. This aspect is considered the most important part of costs due to parasitism. Reduced production is considered to account for 50-66% of the total losses due to nematode or trematode infection in livestock [1,5,126]. However, there are other costs related to parasitism in livestock that need to be taken into account in order to get a more comprehensive appraisal of the total impact of GIN infection on cattle and sheep industry. Other costs due to parasitism include mortality, reduction in fertility, increased sensibility to other pathogens [127,128], and management costs such as prophylactic deworming and clinical treatment in case of symptoms (e.g. anaemia or diarrhoea). Additionally, changes in parasite control strategies might be linked with additional costs or losses: for example, parasitism is unlikely to produce financial losses in cows kept indoors [10,110] but this husbandry system is associated with other production diseases such as lameness or mastitis [129,130]. Thus, although an estimate of the direct financial losses due to GIN infection is a useful first step allowing to appraise the relevance of the disease, it is necessary to integrate those results into a more comprehensive cost-benefit analyses in order to efficiently take economically sound decisions [4,131,132].

CONCLUSION

Overall, this study constitute a synthesis of the work undertaken in Europe on GIN nematode infection in livestock and , the related losses in production and its financial impact in European dairy cattle and meat sheep based on the current available data in literature. This work is to our knowledge the first evaluation of the impact of GIN infection on production in dairy cattle and meat sheep and of its financial consequences at the European scale. Furthermore, we could also identify gaps in knowledge that warrant further research. Finally, in a context of intensification of production and increasing anthelmintic resistance in Europe [2,133], the results presented here can serve as a basis for further more complex cost-benefit analysis or predictive modelling.

Competing interests

The authors declare that they have no competing interests.

Authors' contribution

PT and HH participated in the design of the study. FM performed the literature search and carried out the analysis under PT and HH guidance. FM drafted and finalized the manuscript and all authors contributed to and approved the final version.

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Chapter II - Tables and Figures

Table 1: Variables included in a model to estimate the infection level of gastro-intestinal nematode infection in European dairy cattle and meat sheep and its financial impact on production.

Variable	Assumed distribution	Parameters	Species	Details	Sources
ODR	Gaussian	mean, standard deviation	dairy cattle	Optical density ratio of ELISA against *Ostertagia ostertagi* in milk	Peer-reviewed literature, thesis, conference papers and proceedings
EPG	Negative binomial	mean, aggregation constant *k*	meat sheep	Fecal strongyles egg count in eggs per gram of faeces	Peer-reviewed literature, thesis, conference papers and proceedings
standard deviation of ODR	Gamma	shape, rate	dairy cattle	Measure of variability for ODR at herd and animal level	Peer-reviewed literature
aggregation constant *k* of EPG	Gamma	shape, rate	meat sheep	Measure of variability for EPG at herd and animal level	Peer-reviewed literature, thesis
Days in lactation	Uniform	upper and lower boundaries	dairy cattle	Average number of days in lactation per year for dairy cows	Peer-reviewed literature
Weight at two months	Uniform	upper and lower boundaries	meat sheep	Weight reached by lambs before establishment of GIN infection	Peer-reviewed literature, breeder association reports and websites
Carcass fat trim	Gaussian	mean, standard deviation	Meat sheep	Proportion of lamb weight stored as non-utilizable fat	Peer-reviewed literature, breeder association websites
Carcass weight	-	Point estimate	meat sheep	Average carcass weight of slaughtered lambs in each country	FAO database
Dairy cow population	-	Point estimate	dairy cattle	Number of dairy cows in each country	Eurostat and FAO databases
Lambs slaughtered	-	Point estimate	dairy cattle	Number of lambs slaughtered per year in each country	Eurostat and FAO databases
Proportion of cows grazed	-	Point estimate	dairy cattle	Proportion of cows with access to pasture in each country	Peer-reviewed literature, conference papers and proceedings
Milk production	-	Point estimate	dairy cattle	Quantity of milk produced by dairy cattle in each country in a year	Eurostat and FAO databases
Milk yield	-	Point estimate	Dairy cattle	Average milk yield per cow per year in each country	Eurostat and FAO databases
Meat production	-	Point estimate	meat sheep	Quantity of lamb meat produced in each country in a year	Eurostat and FAO databases
Milk price	-	Point estimate	dairy cattle	Producer price in each country (in Euro/kg)	Eurostat and FAO databases
Lamb meat price	-	Point estimate	meat sheep	Producer price in each country (in Euro/kg)	Eurostat and FAO databases, governmental statistics

Table 2: Parametrization of the measures of variability for the variables used as indicators of gastro-intestinal nematode infection in dairy cattle and meat sheep. For dairy cattle: optical density ratio of ELISA against Ostertagia ostertagi in milk (ODR) through ELISA. For meat sheep: strongyles eggs per gram of faeces determined by faecal egg count (EPG).

Variable	Level	Measure of variability	Distribution	Shape	Rate	No of values	References
ODR	Herd	Standard deviation	Gamma	7.53	35.2	19	[8,10,28,71–78]
ODR	Animal	Standard deviation	Gamma	11.37	47.13	49	[72,110–112]
EPG	Herd	Aggregation constant *k*	Gamma	1.59	1.17	19	[79,82,84–86,88,89,105–107,109,113]
EPG	Animal	Aggregation constant *k*	Gamma	1.94	2.15	301	[82,86,89,93,97,103,104,108]

Table 3: Estimate for the model's parameters in the different bio-economical regions of Europe. For cattle: Optical Density Ratio (ODR) of antibodies level against *Ostertagia ostertagi* in milk; for meat sheep: mean number of strongyles eggs per gram faeces (EPG). Estimates are expressed as 95% uncertainty intervals obtained by Monte-Carlo simulations. For the legend of the regions, see Figure 1.

	Dairy cattle			Meat sheep		
Bio-economical region	**Mean ODR (95%UI)**	**No of herds investigated**	**References**	**Mean EPG (95% UI)**	**No of flocks investigated**	**References**
WHO1/Arctic-Boreal	0.566-0.599	739	[8,75]	336-524	150	[86,87,89]
WHO1/Atlantic	0.758-0.768	6817	[8,10,28,72–74,77]	324-488	286	[9,82–84,88,96,97]
WHO1/Continental	0.606-0.641	652	[8,10]	393-542	307	[9,90,92,98,103]
WHO1/Mediterranean	0.461-0.493	820	[10,71,76]	696-1125	144	[9,81,95,99–102]
WHO2/Continental	0.449-0.611	32	[78]	292-617	49	[80,85,93]
WHO3/Arctic-Boreal	-	-	-	463-701	141	[79,91,94]

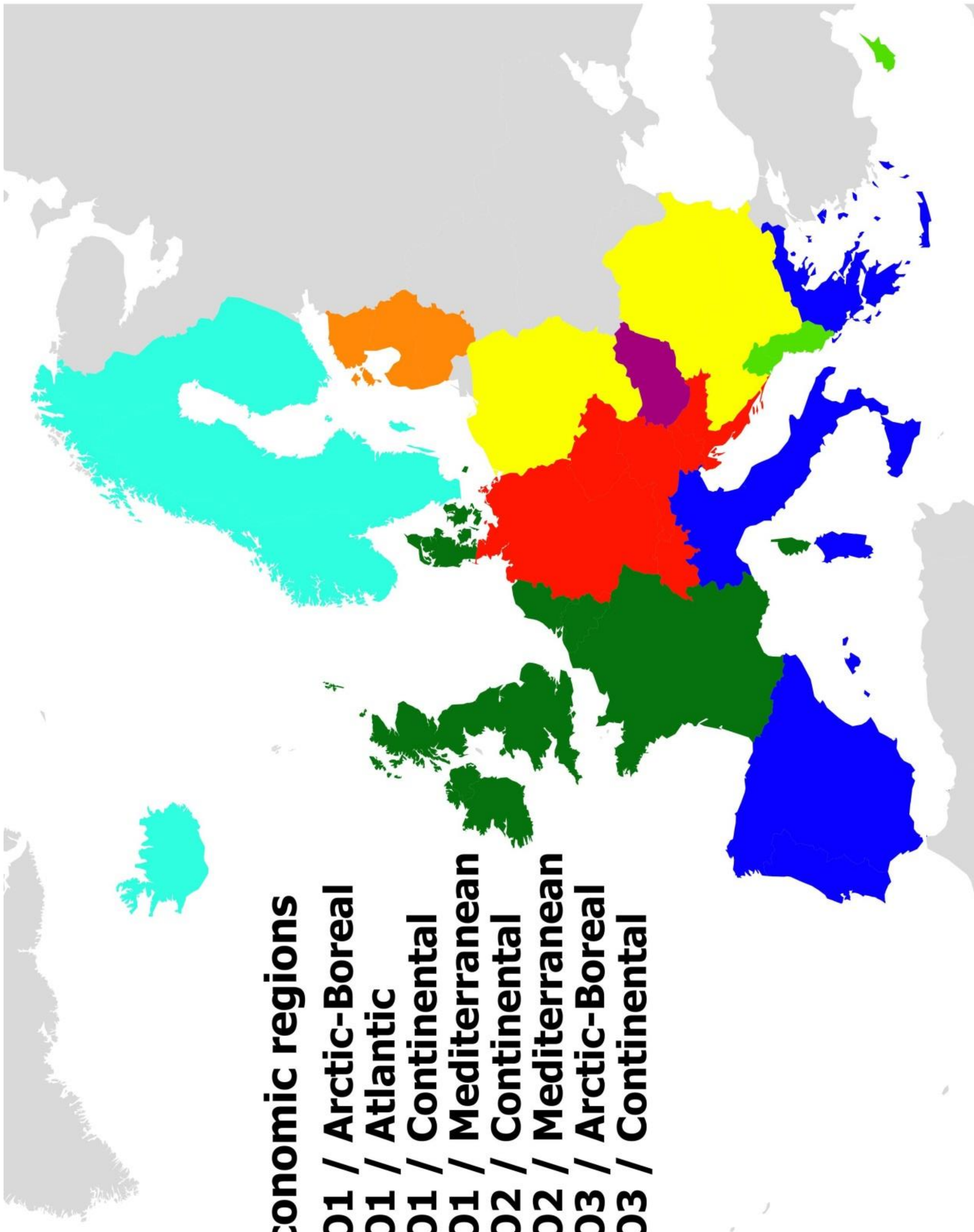

Figure 1: Map of the European countries included in the study and classification in regions according to socio-economic and bio-climatic factors. For a detailed list of countries in each region see Annex A in the supplementary material section.

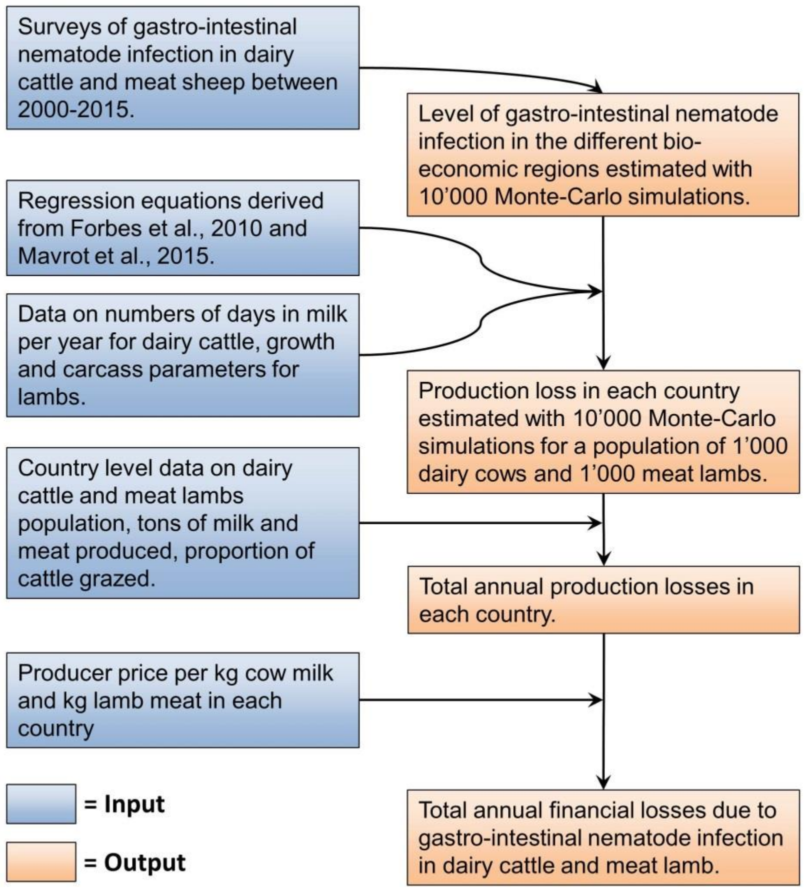

Figure 2: Flow chart of the different steps for the estimation of the financial impact of gastro-intestinal nematode on dairy cattle and meat sheep production in Europe.

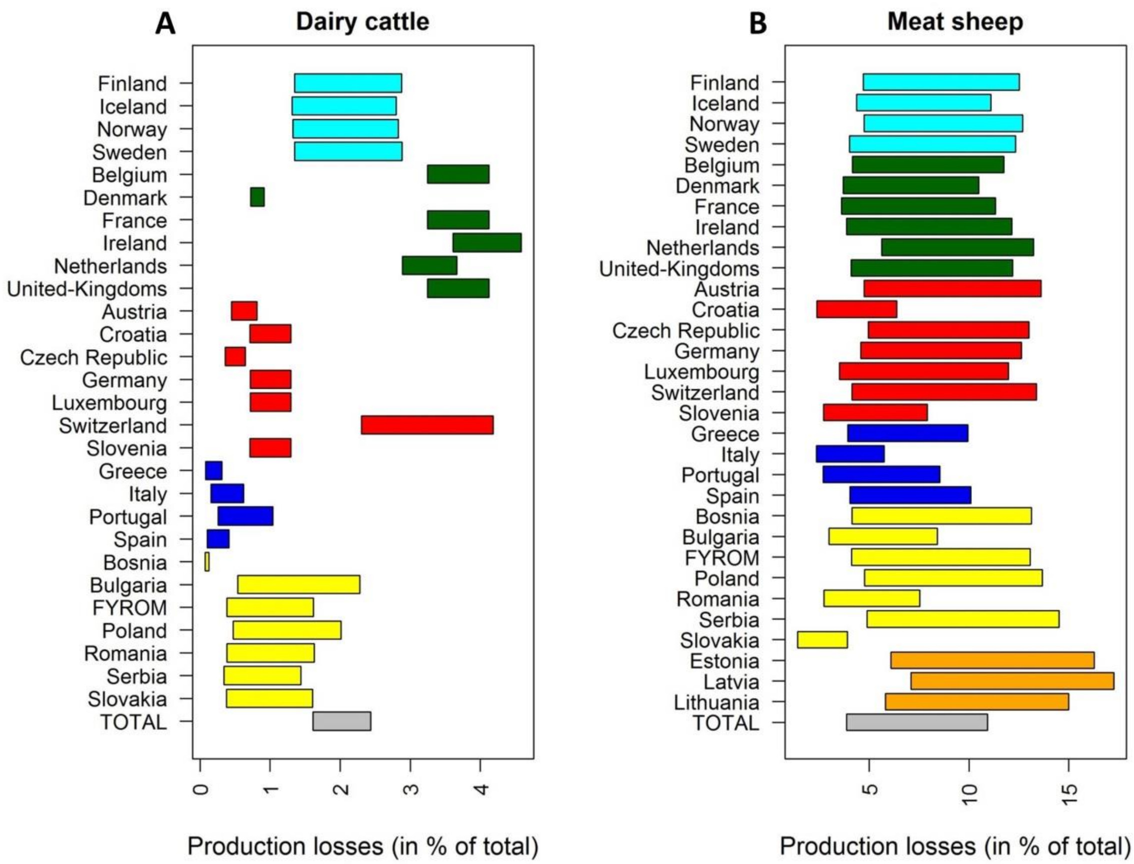

Figure 3: 95% uncertainty interval for the average percentage of total production lost because of gastro-intestinal nematode infection in European dairy cattle and meat sheep. Countries are grouped in bio-economic-regions: Light blue: WHO1/Arctic-boreal; Green: WHO1/Atlantic; Red: WHO1/Continental; Dark blue: WHO1/Mediterranean; Yellow: WHO2/Continental; Orange: WHO3/Arctic-Boreal.

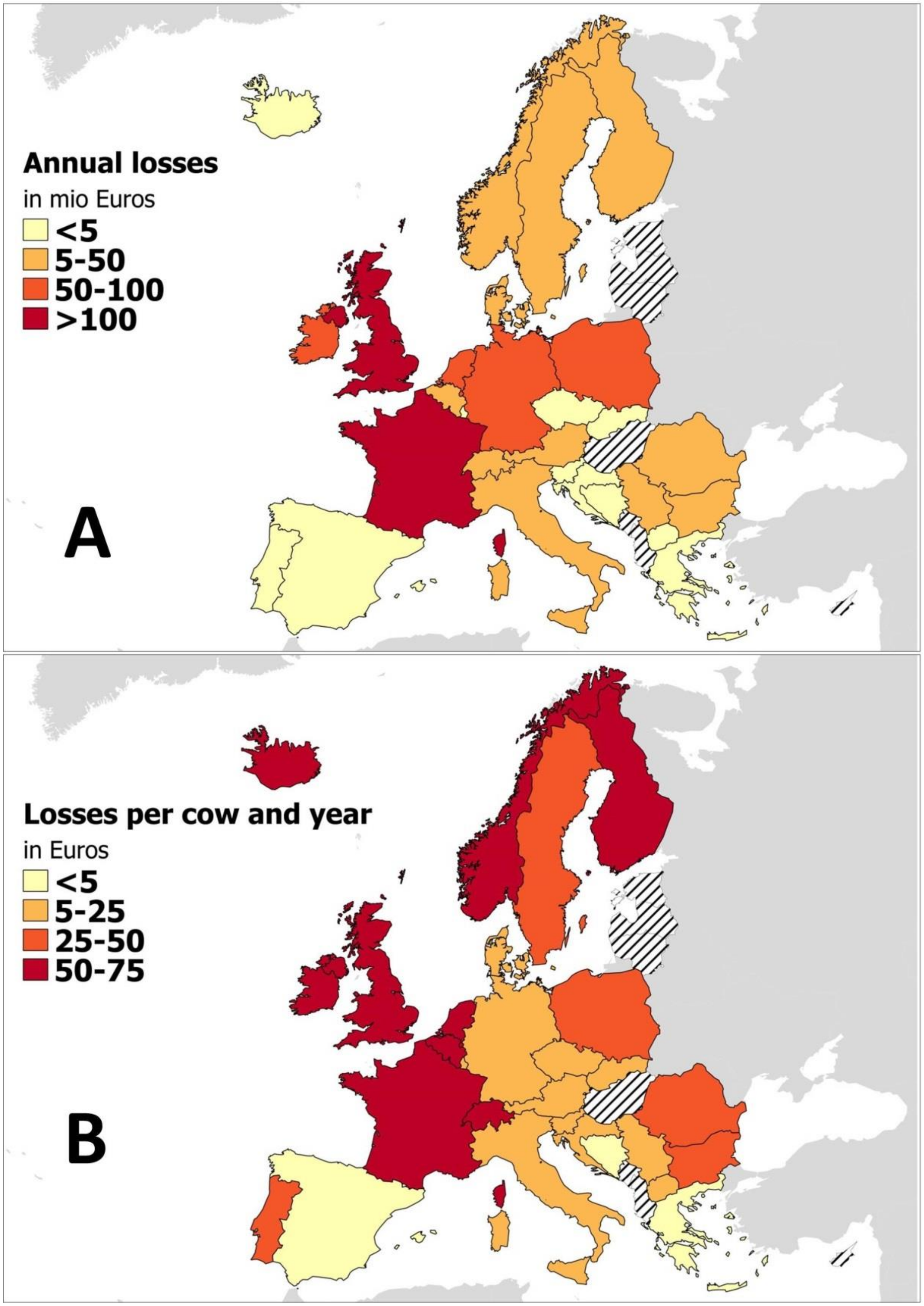

Figure 4: Estimated annual production losses due to gastro-intestinal nematode infection in dairy cattle. Panel A shows the total production losses and panel B represent the estimated losses per dairy cow and per year in the different European countries.

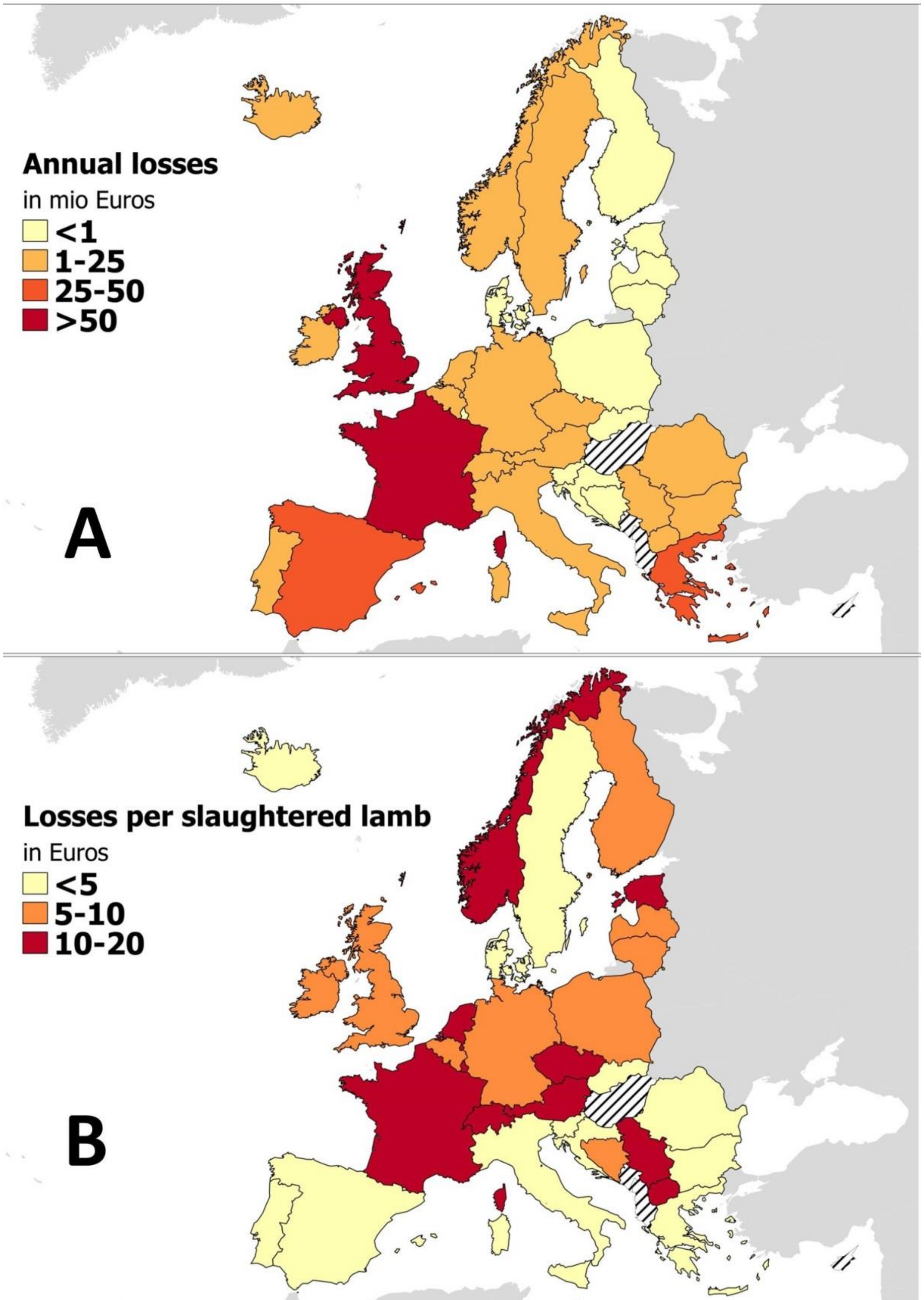

Figure 5: Estimated annual production losses due to gastro-intestinal nematode infection in meat sheep. Panel A shows the total production losses and panel B represent the estimated losses per slaughtered lamb in the different European countries.

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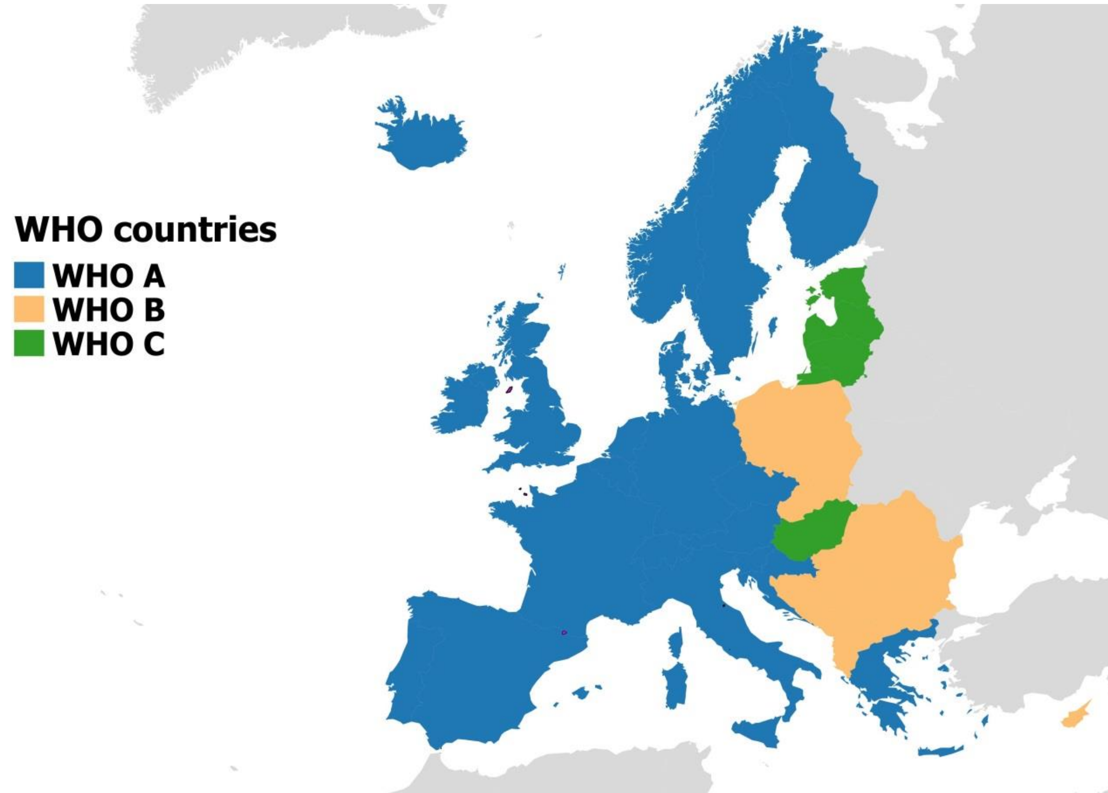
Chapter II – Annex A

Definition of bio-economic regions



Classification of countries according to human mortality as indicator of socio-economic characteristics:

WHO A: very low child and adult mortality

WHO B: low child and adult mortality

WHO C: low child and high adult mortality

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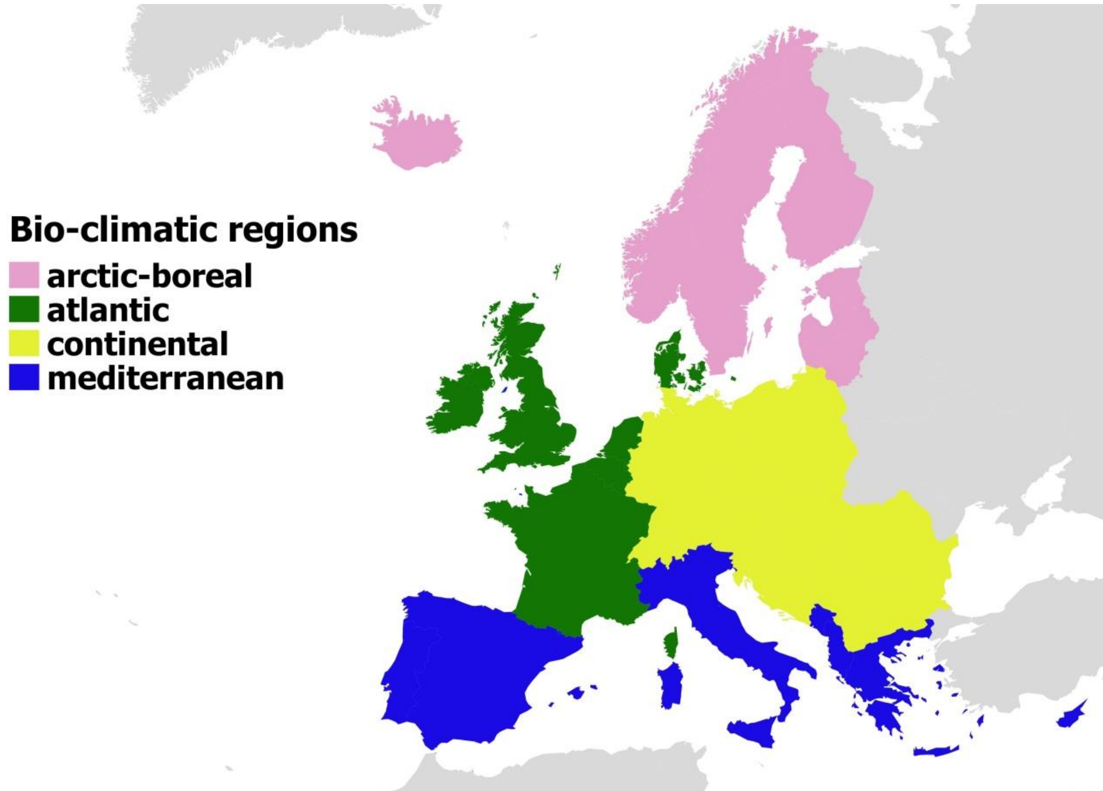

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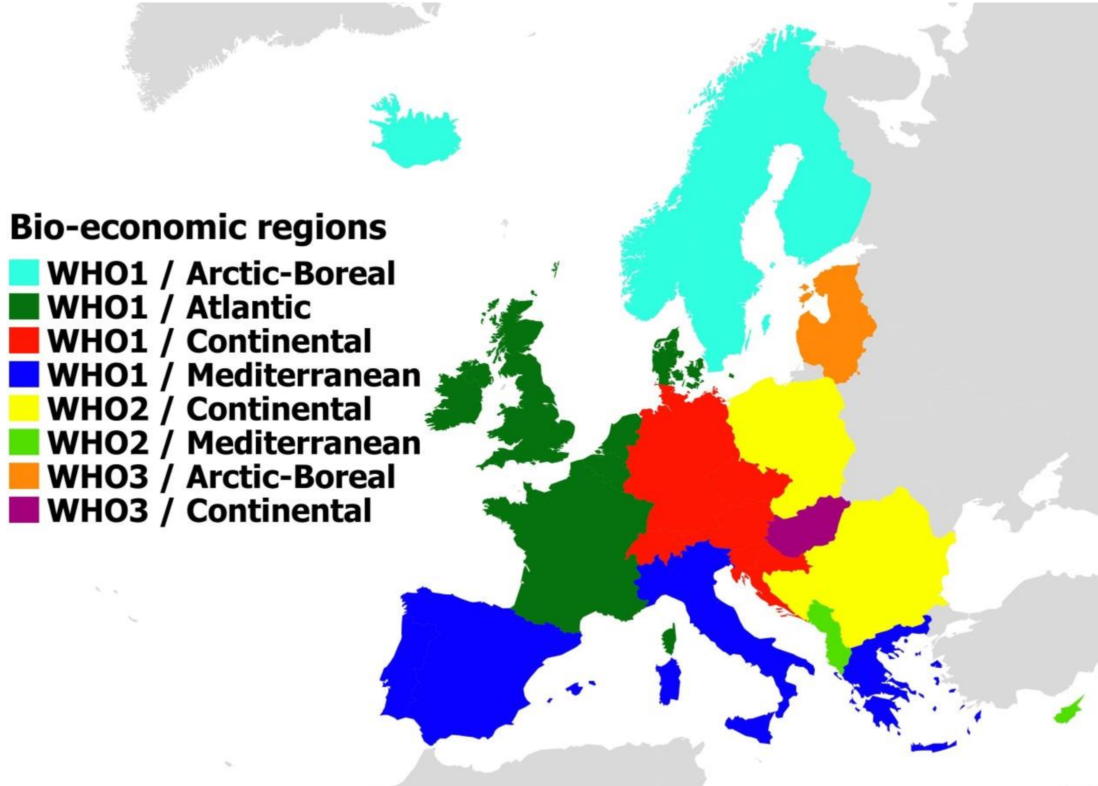

Classification of countries according to socio-economic and bio-climatic characteristics

List of countries:

WHO1 / Arctic-Boreal: Finland, Iceland, Norway, Sweden

WHO1 /Atlantic: Belgium, Denmark, France, Ireland, Netherland, United Kingdom

WHO1 / Continental: Austria, Croatia, Czech Republic, Germany, Liechtenstein, Luxembourg, Slovenia, Switzerland

WHO1 / Mediterranean: Greece, Italy, Malta, Portugal, Spain

WHO2 / Continental: Bosnia, Bulgaria, Former Yugoslavian Republic of Macedonia, Poland, Romania, Serbia, Slovakia

WHO2 / Mediterranean: Albania, Cyprus, Montenegro

WHO3 / Arctic-Boreal: Estonia, Latvia, Lithuania

WHO3 / Continental: Hungary

Chapter II – Annex B

Country-level estimates of financial losses due to nematode infection in European livestock

Financial losses in dairy cattle

Country	Bio-economic region	Dairy cattle population (1'000 heads)	Proportion of animals grazing	Milk production (1'000 tons)	Milk price (€/100 kg)	Ostertagia ostertagi antibody level in milk (optical density ratio)	Estimated loss in milk production (1'000 tons)	95% uncertainty interval
Finland	WHO1 / Arctic-Boreal	281.53	1.00	2'301.00	42.20	0.566-0.599	46.00	31.03-66.27
Iceland	WHO1 / Arctic-Boreal	25.40	1.00	125.09	42.42	0.566-0.599	2.43	1.64-3.51
Norway	WHO1 / Arctic-Boreal	237.57	1.00	1'584.00	60.81	0.566-0.599	31.09	20.97-44.79
Sweden	WHO1 / Arctic-Boreal	347.65	1.00	2'850.00	36.76	0.566-0.599	56.99	38.45-82.10
Belgium	WHO1 / Atlantic	510.65	0.90	3'151.00	28.41	0.758-0.768	111.98	102.35-130.04
Denmark	WHO1 / Atlantic	579.00	0.20	4'880.00	34.18	0.758-0.768	38.53	35.21-44.74
France	WHO1 / Atlantic	3'664.00	0.90	25'116.00	32.96	0.758-0.768	892.54	815.74-1036.46
Ireland	WHO1 / Atlantic	1'035.64	1.00	5'556.00	30.88	0.758-0.768	219.38	200.50-254.76
Netherlands	WHO1 / Atlantic	1'504.00	0.80	11'851.00	31.51	0.758-0.768	374.31	342.10-434.67
United Kingdom	WHO1 / Atlantic	1'800.00	0.90	14'088.00	33.24	0.758-0.768	500.65	457.57-581.38
Austria	WHO1 / Continental	527.39	0.25	3'307.00	34.85	0.606-0.641	19.77	14.78-26.84
Croatia	WHO1 / Continental	184.70	0.40	786.00	32.64	0.606-0.641	7.50	5.61-10.18
Czech Republic	WHO1 / Continental	374.07	0.20	2'736.00	30.66	0.606-0.641	13.08	9.78-17.76
Germany	WHO1 / Continental	4'190.10	0.40	30'301.00	31.59	0.606-0.641	289.85	216.73-393.45
Liechtenstein	WHO1 / Continental	2.99	0.40	13.00	34.02	0.606-0.641	0.13	0.13-0.18
Luxembourg	WHO1 / Continental	44.48	0.40	292.00	30.79	0.606-0.641	2.79	2.09-3.79
Slovenia	WHO1 / Continental	109.07	0.40	602.00	29.65	0.606-0.641	5.75	4.30-7.81
Switzerland	WHO1 / Continental	602.75	0.80	2'583.00	47.95	0.606-0.641	79.57	59.50-108.01
Greece	WHO1 / Mediterranean	130.00	0.15	757.00	42.63	0.461-0.493	1.34	0.59-2.36
Italy	WHO1 / Mediterranean	1'754.98	0.30	11'299.00	37.50	0.461-0.493	40.11	17.55-70.46
Portugal	WHO1 / Mediterranean	241.95	0.50	1'919.00	31.59	0.461-0.493	11.35	4.97-19.94
Spain	WHO1 / Mediterranean	797.89	0.20	6'488.00	30.01	0.461-0.493	15.35	6.72-26.97
Bosnia	WHO2 / Continental	250.00	0.05	674.00	29.28	0.449-0.611	0.52	0.49-0.88
Bulgaria	WHO2 / Continental	313.18	0.97	1'126.00	33.57	0.449-0.611	15.19	6.06-25.67
FYROM	WHO2 / Continental	123.00	0.60	349.77	31.60	0.449-0.611	3.36	1.34-5.67
Poland	WHO2 / Continental	2'446.14	0.75	12'414.00	28.38	0.449-0.611	147.72	58.98-249.65

Financial losses in dairy cattle

Country	Percent of total production lost	95% uncertainty interval	Total financial losses (mio €)	95% uncertainty interval	Loss per cow (€)	95% uncertainty interval	Loss per grazed cow (€)	95% uncertainty interval
Finland	2.00	1.35-2.88	19.41	13.10-27.96	68.95	46.52-99.33	68.95	46.52-99.33
Iceland	1.95	1.31-2.80	1.03	0.70-1.49	40.65	27.42-58.55	40.65	27.42-58.55
Norway	1.96	1.32-2.83	18.91	12.76-27.24	79.59	53.69-114.65	79.59	53.69-114.65
Sweden	2.00	1.35-2.88	20.95	14.13-30.18	60.26	40.65-86.81	60.26	40.65-86.81
Belgium	3.55	3.25-4.13	31.82	29.08-36.95	62.31	56.95-72.35	69.23	63.27-80.39
Denmark	0.79	0.72-0.92	13.17	12.04-15.29	22.75	20.79-26.41	113.73	103.94-132.07
France	3.55	3.25-4.13	294.15	268.84-341.58	80.28	73.37-93.22	89.20	81.52-103.58
Ireland	3.95	3.61-4.59	67.74	61.91-78.66	65.41	59.78-75.95	65.41	59.78-75.95
Netherlands	3.16	2.89-3.67	117.94	107.79-136.96	78.42	71.67-91.06	98.02	89.59-113.83
United Kingdom	3.55	3.25-4.13	166.42	152.10-193.25	92.46	84.50-107.36	102.73	93.89-119.29
Austria	0.60	0.45-0.81	6.89	5.15-9.35	13.07	9.77-17.73	52.26	39.08-70.94
Croatia	0.95	0.71-1.30	2.45	1.83-3.32	13.26	9.91-17.99	33.14	24.78-44.99
Czech Republic	0.48	0.36-0.65	4.01	3.00-5.45	10.72	8.02-14.56	53.62	40.09-72.78
Germany	0.96	0.72-1.30	91.57	68.47-124.30	21.85	16.34-29.67	54.64	40.85-74.16
Liechtenstein	1.03	1.02-1.40	0.05	0.04-0.06	15.19	15.01-20.61	37.97	37.54-51.54
Luxembourg	0.96	0.72-1.30	0.86	0.64-1.17	19.34	14.46-26.25	48.35	36.16-65.64
Slovenia	0.96	0.71-1.30	1.71	1.28-2.31	15.64	11.69-21.22	39.09	29.23-53.06
Switzerland	3.08	2.30-4.18	38.16	28.53-51.79	63.30	47.33-85.93	79.13	59.17-107.41
Greece	0.18	0.08-0.31	0.57	0.25-1.01	4.41	1.93-7.74	29.37	12.86-51.60
Italy	0.35	0.16-0.62	15.04	6.58-26.42	8.57	3.75-15.05	28.57	12.50-50.18
Portugal	0.59	0.26-1.04	3.59	1.57-6.30	14.82	6.49-26.03	29.64	12.97-52.06
Spain	0.24	0.10-0.42	4.61	2.02-8.09	5.77	2.53-10.14	28.87	12.64-50.71
Bosnia	0.08	0.07-0.13	0.15	0.14-0.26	0.61	0.57-1.03	12.16	11.45-20.55
Bulgaria	1.35	0.54-2.28	5.10	2.04-8.62	16.28	6.50-27.51	16.78	6.70-28.36
FYROM	0.96	0.38-1.62	1.06	0.42-1.79	8.62	3.44-14.57	14.37	5.74-24.28
Poland	1.19	0.48-2.01	41.93	16.74-70.86	17.14	6.84-28.97	22.85	9.12-38.62

Financial losses in dairy cattle

Country	Bio-economic region	Dairy cattle population (1'000 heads)	Proportion of animals grazing	Milk production (1'000 tons)	Milk price (€/100 kg)	Ostertagia ostertagi antibody level in milk (optical density ratio)	Estimated loss in milk production (1'000 tons)	95% uncertainty interval
Romania	WHO2 / Continental	1'170.00	0.60	4'075.00	30.62	0.449-0.611	39.33	15.70-66.47
Serbia	WHO2 / Continental	463.00	0.60	1'510.00	26.51	0.449-0.611	12.87	5.14-21.76
Slovakia	WHO2 / Continental	154.11	0.60	928.00	27.52	0.449-0.611	8.84	3.53-14.94
TOTAL		**23'865.24**		**153'661.86**			**2'988.34**	**2'479.57-3'751.47**

Financial losses in dairy cattle

Country	Percent of total production lost	95% uncertainty interval	Total financial losses (mio €)	95% uncertainty interval	Loss per cow (€)	95% uncertainty interval	Loss per grazed cow (€)	95% uncertainty interval
Romania	0.97	0.39-1.63	12.04	4.81-20.35	10.29	4.11-17.39	17.15	6.85-28.99
Serbia	0.85	0.34-1.44	3.41	1.36-5.77	7.37	2.94-12.46	12.29	4.91-20.76
Slovakia	0.95	0.38-1.61	2.43	0.97-4.11	15.78	6.30-26.67	26.30	10.50-44.45
TOTAL	**1.94**	**1.61-2.44**	**987.15**	**818.28-1'240.89**				

Financial losses in meat sheep

Country	Bio-economic region	Lambs slaughtered (1'000 heads)	Carcass weight (kg)	Baseline carcass weight (kg)	Meat production (1'000 tons)	Milk price (€/100 kg)	Faecal egg count (eggs per gram)	Estimated loss in sheep meat production (1'000 tons)	95% uncertainty interval
Finland	WHO1 / Arctic-Boreal	48.50	19.59	8.0-8.5	0.95	301.76	336-524	0.09	0.04-0.12
Iceland	WHO1 / Arctic-Boreal	583.32	17.01	8.0-8.5	9.921	321.03	336-524	0.85	0.43-1.10
Norway	WHO1 / Arctic-Boreal	1'133.89	20.09	8.0-8.5	22.777	603.33	336-524	2.25	1.08-2.89
Sweden	WHO1 / Arctic-Boreal	261.57	19.23	8.0-8.5	5.03	210.79	336-524	0.48	0.20-0.62
Belgium	WHO1 / Atlantic	130.00	19.00	8-9	2.47	509.90	324-488	0.22	0.10-0.39
Denmark	WHO1 / Atlantic	80.13	16.82	8-9	1.348	320.72	324-488	0.11	0.05-0.14
France	WHO1 / Atlantic	6'265.98	18.17	8-9	113.846	608.98	324-488	9.83	4.10-12.89
Ireland	WHO1 / Atlantic	2'716.00	19.77	8-9	53.7	329.40	324-488	5.02	2.07-6.52
Netherlands	WHO1 / Atlantic	584.50	22.24	8-9	13.002	536.44	324-488	1.34	0.73-1.72
United Kingdom	WHO1 / Atlantic	13'746.00	20.01	8-9	275	438.03	324-488	25.57	11.27-33.52
Austria	WHO1 / Continental	289.53	22.81	8-9	6.604	431.73	393-542	0.70	0.31-0.90
Croatia	WHO1 / Continental	166.70	12.00	8-9	2	637.54	393-542	0.10	0.05-0.13
Czech Republic	WHO1 / Continental	131.37	21.14	8-9	2.777	452.24	393-542	0.28	0.14-0.36
Germany	WHO1 / Continental	1'813.53	20.15	8-9	36.544	305.18	393-542	3.65	1.67-4.61
Luxembourg	WHO1 / Continental	2.61	18.77	8-9	0.049	649.35	393-542	0.00	0.00-0.01
Slovenia	WHO1 / Continental	104.64	13.43	8-9	1.405	432.02	393-542	0.09	0.04-0.11
Switzerland	WHO1 / Continental	227.41	22.00	8-9	5.002	790.51	393-542	0.52	0.21-0.67
Greece	WHO1 / Mediterranean	7'905.00	11.39	6.5-7.5	90	331.74	696-1125	7.08	3.53-8.95
Italy	WHO1 / Mediterranean	5'089.61	8.95	6.5-7.5	45.558	722.56	696-1125	2.07	1.07-2.62
Portugal	WHO1 / Mediterranean	1'668.00	10.51	6.5-7.5	17.524	469.08	696-1125	1.19	0.47-1.49
Spain	WHO1 / Mediterranean	10'518.67	11.60	6.5-7.5	121.999	327.20	696-1125	9.92	4.91-12.31
Bosnia	WHO2 / Continental	133.91	16.95	6.5-7.5	2.27	370.25	292-617	0.22	0.09-0.30
Bulgaria	WHO2 / Continental	1'218.99	11.32	6.5-7.5	13.796	461.66	292-617	0.87	0.41-1.16
FYROM	WHO2 / Continental	329.00	17.02	6.5-7.5	5.6	595.78	292-617	0.55	0.23-0.73
Poland	WHO2 / Continental	48.79	18.45	6.5-7.5	0.9	398.27	292-617	0.09	0.04-0.12
Romania	WHO2 / Continental	6'448.00	10.62	6.5-7.5	68.504	336.44	292-617	3.93	1.87-5.16

Financial losses in meat sheep

Country	Percent of total production lost	95% uncertainty interval	Total financial losses (mio €)	95% uncertainty interval	Loss per sheep(€)	95% uncertainty interval
Finland	9.73	4.71-12.52	0.28	0.13-0.36	5.75	2.78-7.40
Iceland	8.54	4.36-11.08	2.72	1.39-3.53	4.66	2.38-6.05
Norway	9.87	4.73-13.69	13.57	6.51-17.44	11.97	5.74-15.38
Sweden	9.49	4.00-12.33	1.01	0.42-1.31	3.85	1.62-5.00
Belgium	9.09	4.16-11.76	1.14	0.52-1.48	8.81	4.03-11.39
Denmark	8.13	3.69-10.49	0.35	0.16-0.45	4.39	1.99-5.66
France	8.64	3.60-11.32	59.87	24.95-78.49	9.55	3.98-12.53
Ireland	9.34	3.86-12.14	16.52	6.83-21.47	6.08	2.52-7.91
Netherlands	10.32	5.62-13.24	7.20	3.92-9.24	12.31	6.70-15.80
United Kingdom	9.30	4.10-12.19	111.99	49.38-146.81	8.15	3.59-10.68
Austria	10.60	4.74-13.62	3.02	1.35-3.88	10.44	4.67-13.41
Croatia	5.04	2.37-6.38	0.64	0.30-0.81	3.86	1.81-4.88
Czech Republic	10.19	4.94-13.00	1.28	0.62-1.63	9.74	4.73-12.43
Germany	9.98	4.57-12.63	11.13	5.10-14.08	6.14	2.81-7.76
Luxembourg	9.21	3.50-11.98	0.03	0.01-0.04	11.22	4.26-14.61
Slovenia	6.12	2.70-7.91	0.37	0.16-0.48	3.55	1.57-4.59
Switzerland	10.38	4.14-13.38	4.10	1.64-5.29	18.04	7.19-23.27
Greece	7.87	3.92-9.94	23.50	11.70-29.68	2.97	1.48-3.76
Italy	4.54	2.35-5.75	14.96	7.72-18.94	2.94	1.52-3.72
Portugal	6.78	2.69-8.53	5.58	2.21-7.01	3.34	1.32-4.20
Spain	8.13	4.03-10.09	32.44	16.08-40.28	3.08	1.53-3.83
Bosnia	9.91	4.12-13.12	0.83	0.35-1.10	6.22	2.59-8.24
Bulgaria	6.33	6.33-8.41	4.03	1.90-5.36	3.31	1.56-4.40
FYROM	9.87	4.11-13.07	3.29	1.37-4.36	10.01	4.16-13.25
Poland	10.41	4.76-13.68	0.37	0.17-0.49	7.65	3.50-10.05
Romania	5.74	2.74-7.54	13.22	6.31-17.38	2.05	0.98-2.69

Financial losses in meat sheep

Country	Bio-economic region	Lambs slaughtered (1'000 heads)	Carcass weight (kg)	Baseline carcass weight (kg)	Meat production (1'000 tons)	Milk price (€/100 kg)	Faecal egg count (eggs per gram)	Estimated loss in sheep meat production (1'000 tons)	95% uncertainty interval
Serbia	WHO2 / Continental	1'107.69	20.20	6.5-7.5	22.373	450.44	292-617	2.46	1.09-3.25
Slovakia	WHO2 / Continental	117.65	8.50	6.5-7.5	1	168.72	292-617	0.03	0.01-0.04
Estonia	WHO3 / Arctic-Boreal	39.21	17.85	5.0-5.5	0.7	410.41	463-701	0.09	0.04-0.11
Latvia	WHO3 / Arctic-Boreal	33.07	20.53	5.0-5.5	0.679	311.42	463-701	0.09	0.05-0.12
Lithuania	WHO3 / Arctic-Boreal	26.76	15.02	5.0-5.5	0.402	368.63	463-701	0.05	0.02-0.06
TOTAL		**62'970.04**			**943.73**			**79.74**	**36.35-103.02**

Financial losses in meat sheep

Country	*Percent of total production lost*	*95% uncertainty interval*	*Total financial losses (mio €)*	*95% uncertainty interval*	*Loss per sheep(€)*	*95% uncertainty interval*
Serbia	10.99	4.88-14.52	11.07	4.92-14.63	10.00	4.44-13.21
Slovakia	2.96	2.96-3.90	0.05	0.02-0.07	0.42	0.20-0.56
Estonia	12.99	6.08-16.28	0.37	0.17-0.47	9.52	4.45-11.93
Latvia	13.61	7.09-17.27	0.29	0.15-0.37	9.24	4.53-11.04
Lithuania	11.84	5.80-14.99	0.18	0.09-0.22	6.56	3.21-8.30
TOTAL			**372.47**	**156.56-447.15**		

Chapter II – Annex C

Supplementary material

Table 1: Synopsis of anthelmintics usage for sheep and dairy cattle in different European countries between 2000 and 2015.

Country	Species	Bio-economical region	No deworming/total	Percent deworming	95% uncertainty interval	References
Switzerland	Sheep	WHO1/Continental	109/109	100.00	95.76-100	[1]
Slovakia	Sheep	WHO2/Continental	43/43	100.00	89.79-100	[2]
Germany	Sheep	WHO1/Continental	28/28	100.00	84.98-100	[3]
Spain	Sheep	WHO1/Mediterranean	349/352	99.15	97.32-99.78	[4–6]
Norway	Sheep	WHO1/Arctic-Boreal	581/587	98.98	97.67-99.58	[7]
United Kingdom	Sheep	WHO1/Atlantic	913/970	94.12	92.41-95.48	[8–10]
Greece	Sheep	WHO1/Mediterranean	830/1017	81.61	79.06-83.92	[11–13]
Estonia	Sheep	WHO3/Arctic-Boreal	30/45	66.67	50.95-79.56	[14]
Total	**Sheep**	**All**	**2883/3151**	**91.50**	**90.45-92.43**	-
France	Cattle	WHO1/Atlantic	42/47	89.36	76.11-96.02	[15]
Netherlands	Cattle	WHO1/Atlantic	74/86	86.05	76.50-92.28	[16]
Ireland	Cattle	WHO1/Atlantic	208/302	68.87	63.27-73.99	[17]
United Kingdom	Cattle	WHO1/Atlantic	122/302	40.40	34.86-46.19	[17]
Belgium	Cattle	WHO1/Atlantic	591/1486	39.77	37.28-42.32	[17–20]
Germany	Cattle	WHO1/Continental	64/355	18.03	14.25-22.51	[17,20]
Sweden	Cattle	WHO1/Arctic-Boreal	52/297	17.51	13.46-22.42	[17,20,21]
Spain	Cattle	WHO1/Mediterranean	43/256	16.80	12.54-22.07	[22]
Total	**Cattle**	**All**	**1215/3164**	**38.40**	**36.71-40.12**	-

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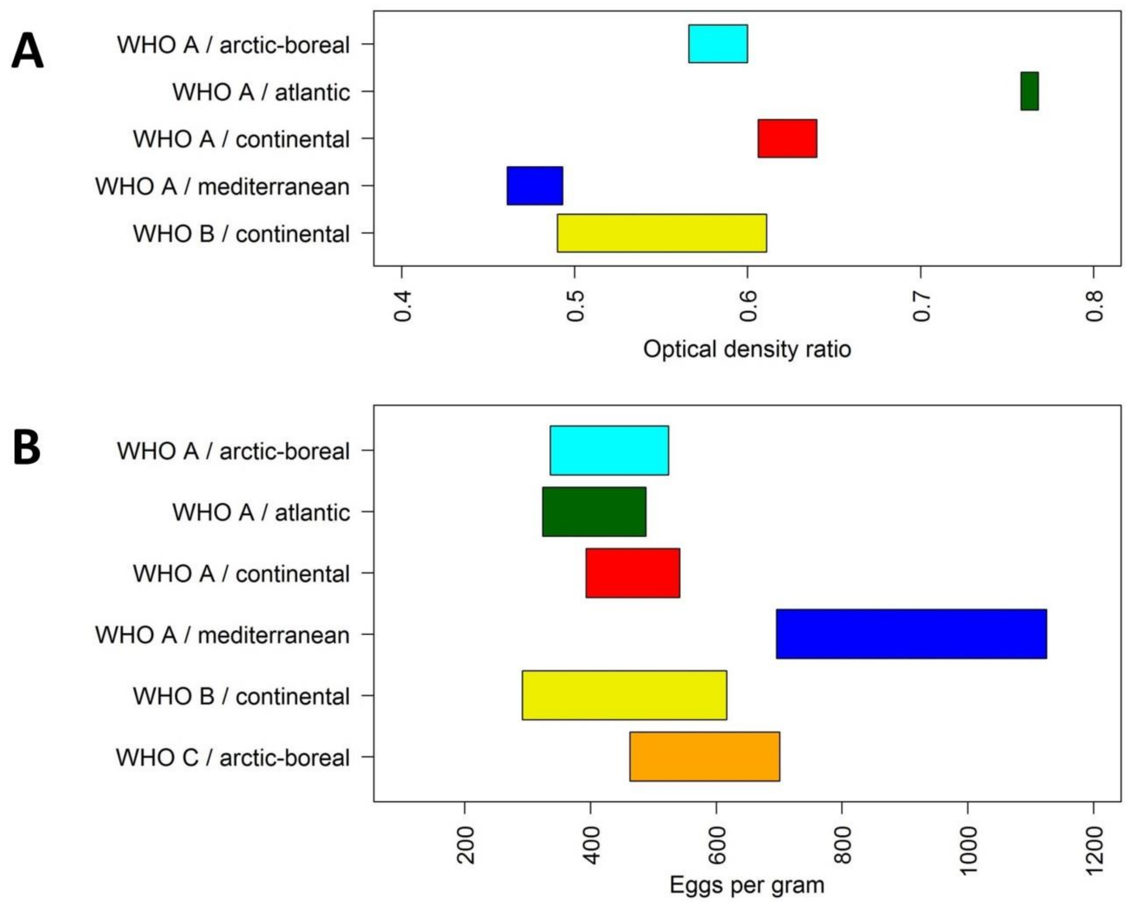

Figure 1: Graphical representation of the 95% uncertainty intervals estimated for nematode infection levels in the different bio-economic regions of Europe. A: Infection levels in dairy cows expressed as optical density ratio for anti-*Ostertagia ostertagi* antibody level in milk. B Infection levels in meat lambs expressed as eggs per gram of faeces.

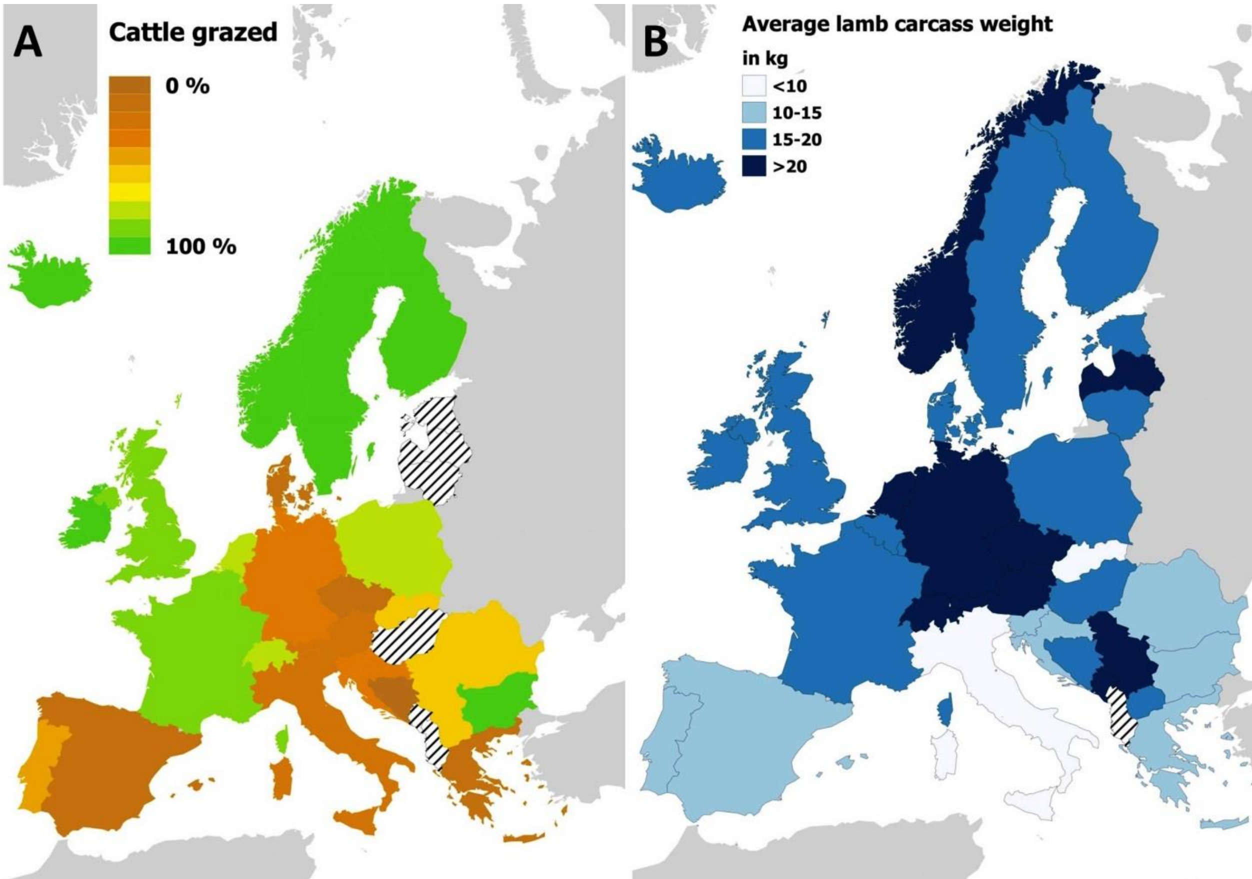

Figure 2: Management parameters used for the estimation of financial losses due to nematode infection in dairy cattle and meat sheep in Europe. A: Proportion of dairy cattle grazed at least a part of the year in the different European countries. B: Average carcass weight of lambs at slaughter in the different European countries.

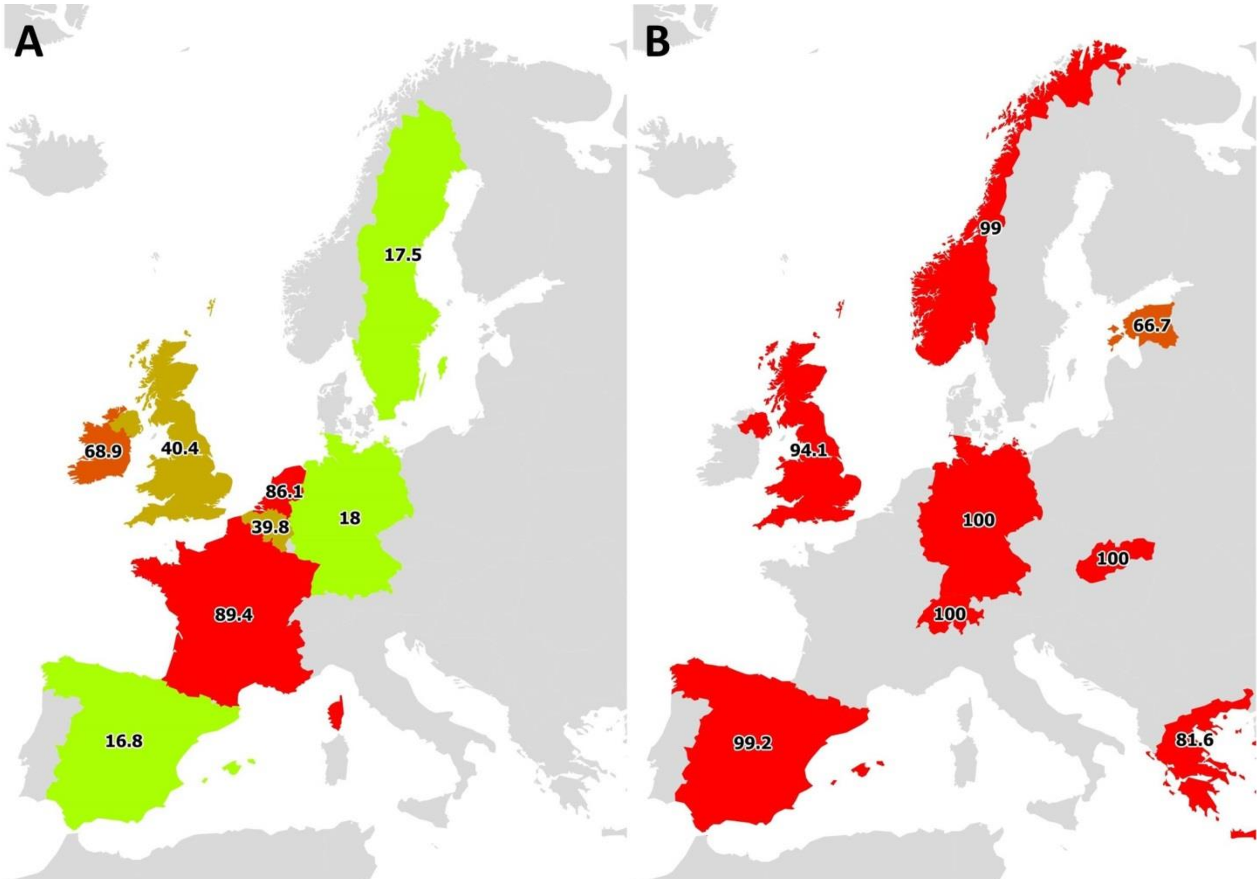

Figure 3: Reported proportion of farmers using anthelmintic for dairy cows (A) and sheep (B) in studies published between 2000 and 2015 in different European countries.

CHAPTER III

Development of Haemonchus contortus on Swiss alpine pastures and its relationship with weather conditions

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ABSTRACT

Haemonchus contortus is one the most important gastro-intestinal nematode of sheep in temperate regions. Although control of H. contortus is usually successfully achieved with prophylactic or therapeutic treatment, the increase in prevalence of anthelmintic resistances emphasizes the need for alternative control strategies. In addition, climate change is expected to modify the geographical distribution of the parasite by influencing its development and survival outside the host.

In this study, we modelled the development of *H. contortus* on Swiss alpine pastures in relation with weather variables using generalized additive mixed model procedures. In 22 experimental trials conducted between 2012 and 2014, we transferred faeces of sheep monoinfected with *H. contortus* on alpine pastures at different altitudes and monitored the amount of infective larvae retrieved in grass at weekly intervals. In parallel, we recorded temperature, humidity and rainfall during each trial.

Our results indicate that *H. contortus* can develop into third-stage larvae at altitudes as high as 2000 meters above sea level in Switzerland. There was a non-linear, non-monotonic relationship between the numbers of larvae retrieved on pasture and the total cumulative temperature since the start of each trial. Additionally, other parameters such as total rainfall and weather conditions at the beginning of each trial were decisive for the development of the infective larvae. The results presented here will serve as a basis for predicting future spatial and temporal changes in the epidemiology of haemonchosis and help develop sustainable alternatives to anthelmintics for the control of the parasite.

Keywords: *Haemonchus contortus*, free-living stage, climate, environment, generalized additive mixed model

INTRODUCTION

Many parasitic helminth life cycles have a free-living stage. Gastro-intestinal helminths of herbivores have developed different strategies in order to develop outside their definitive host. Some infect one or more intermediate hosts (e.g. liver flukes), whereas other parasites have evolved to survive in the soil and the herbage (many nematodes) until they are ingested by their definitive host. For those helminths, survival and development in the environment is a key parameter for their ability to infect new definitive hosts and perpetuate the cycle ([1]). In particular, weather and climate related parameters such as humidity or temperature are considered important drivers of the development of parasites outside its host [2, 3].

In temperate and tropical regions, *Haemonchus contortus* is an important parasite of sheep [4, 5]. It affects the host by sucking blood through the abomasum wall, causing anaemia which can be fatal in case of severe infection [1]. Although control of the worm population is usually achieved using anthelmintics, increasing resistance to anthelmintics as well as growing interest in biological production have led to the investigation of alternative worm control methods. These include rotational or evasive grazing strategies, vaccination, or biological control [4]. In particular, it has been proposed to exploit seasonal patterns in climate in order to control the population of *H. contortus* in a herd and reduce the infection pressure induced by the parasite [6–8].

On a broader perspective, the geographical distribution of *H. contortus* is also dependent on environmental and climatic conditions along with management practices [5]. Climate change is hypothesized to be responsible for the northern spread of the parasite observed in recent years [9, 10]. In this context, a better knowledge of the effect of the different meteorological parameters on the ecology of the parasite can help predicting further changes in the distribution of *H. contortus* and implement suitable control strategies.

In the present study, we aim at documenting the development of *H. contortus* on Swiss alpine pasture at different altitudes by conducting a series of experimental trials over three years. Furthermore, we use an additive mixed model approach which allows us to investigate the combined effect of several standard meteorological indicators on larval development of *H. contortus*. The results will increase the knowledge of the ecology of *H. contortus* in temperate regions and provide a basis for predictive modelling of the parasite occurrence and spread as well as proposing targeted management measures.

MATERIAL AND METHODS

Experimental trials: In order to document the development of *H. contortus* on in a natural environment, we transferred faeces of infected sheep to pasture plots at various altitudes and measured how many infective larvae of the parasite were found in the grass during the following month.

Experiment locations and settings: The trials were conducted in four locations in the canton of Grison in the Swiss Alps. The maximum geographic distance between the four experimental locations was 70 km. The 4 locations were situated at an altitude of 500, 1000, 1500 and 2000 meters above sea level respectively. At each location, a suitable area of pasture that was relatively flat and had not been grazed upon by sheep during the previous years was identified and fenced. Each experimental trial was run for four weeks. At each location, the experiments were run on four grass plots in parallel. The plots were circular areas with a radius of 30 cm and were separated into four quadrants of equal surface. The plots were further separated in an inner (<15 cm radius) and an outer zone (>15 cm radius, Fig. 1).

Experimental protocol: The development of third-stage larvae of *H. contortus* was documented described by Hertzberg et al. [11] for *Cooperia oncophora.* Twenty-four to 48 hours prior to the start of each experiment, we collected faeces from a sheep monoinfected with *H. contortus* and kept at the Institute of Parasitology of the University of Zürich [12]. The quantity of *H. contortus* eggs in the faeces was determined by faecal egg count using a modified McMaster method [13]. The collected faeces were kept in plastic jars in the dark at 5-10°C until required. At the beginning of the experiment (day 0), the faeces were brought to the experimental plots and 50 grams of faeces were deposited at the centre of each four plots at each location. The plots were protected from intrusion with a fence and a bird net. On each plot, the grass was collected in one of the four quadrants on days 7, 14, 21 and 28, following a clockwise order on the plot (Fig. 1). In addition, at each location, the starting quadrant was different for each of the four plots. This was done to avoid bias caused by external factors such as slope or wind. Herbage larval count was then performed on the collected grass as previously described [14]. Thus for each experiment, 4x4 larval counts were obtained (four weekly counts on four plots), in addition, each count was composed of a subtotal for the inner and outer zone. Finally, at the end of each trial, new plots were established for the next experiment with a distance of at least 100 cm to the plots previously used in order to avoid contamination from previous trials.

Weather monitoring: At each trial location, two data loggers (Tinytag® Ultra and Talk, model TGU-4500 and TK-4023, INTAB Benelux, The Netherlands) and a rain gauge (MeteoFrance SPIEA 1650-02, Benoit, France) were used to measure the shade air temperature, and soil temperature at the ground level, relative humidity and rainfall during each trial. Temperature and humidity were recorded every 30 minutes. Rainfall was measured weekly simultaneously to grass collection.

Data analysis: We modelled the number of *H. contortus* larvae retrieved at each herbage larval count as a negative binomial outcome variable depending on different predictors using a generalized additive mixed modelling approach. Additive models have the advantage of enabling the investigation of non-linear, non-monotonic relationship between variables. For example, it is documented that larval development of *H. contortus* reaches its maximum within 25-35°C but decreases at temperatures above or below this interval [2, 15]. Thus, we investigated a possible non-linear, non-monotonic effect of temperature, but also of rainfall. Mixed models allow us to add a random component to our analysis. Here, we defined the locations where we conducted the trials as a random-effect variable in our analysis.

Variables included in our analysis are shown in table 1. We used air temperature, air humidity and rainfall as weather variables and faecal egg count, week, and location as additional variables. Weather variables were either the average value measured during the week preceding each herbal larval count or the average value during the first week after the start of the experiment. In addition, for air temperature and rainfall, we also included variables summarizing the total rainfall and temperature since the start of each experiment. Total temperature was defined as the sum of the average daily temperatures and served as a proxy for the amount of accumulated heat similarly to degree-days used in previous studies [16, 17].

Model construction was done using forward selection and using Aikake Information Criterion (AIC) to select the best model. In addition, we also investigated models including interaction terms between temperature, rainfall and humidity. All analysis were carried on using the R statistical program [18] and the R packages “MASS” and “car” [19, 20].

RESULTS

In total, we ran 22 experimental trials 2012 and 2014: seven at 500 and 1000 meters, six at 1500 meters and two at 2000 meters (Fig. 2) for a total of 328 larval counts. At 1500 meters, the experiment was stopped on three occasions at days 21, 14 and 28 respectively because of snowfall. Infective larvae of *H. contortus* were recovered during all trials and at all altitudes. Weekly rainfall during the experiments ranged from 0 to 108 mm with a maximum rainfall over a one-month period of 201mm. Average air temperature during a week ranged from 4 to 24°C but peak air temperatures up to 35°C were reached during the hottest period of some days. Moreover, peak soil temperatures above 40°C were recorded during seven weeks at 500, 1000 and 2000 meters altitude. Development of infective larvae was observed under almost every condition, even after weeks with average daily minimal and maximal soil temperatures below 10°C or above 40°C, respectively. Altogether, the median number of larvae recovered per million eggs of *H. contortus* deposited on each plot was 0 on day 7 (interquartile range [IQR]: 0-29), 32 on day 14 (IQR: 0-283), 472 on day 21 (IQR: 82-1562) and 1046 on day 28 (IQR: 182-2423). The highest herbage larval count was recorded at 500m altitude on day 21 after depositing the faeces and amounted to 4017 larvae per million eggs (approximatively 1.6% of the quantity of deposited eggs). Fig. 3 shows the number of larvae recovered per million eggs at different altitudes and on different days. In addition, 94% of the larvae were recovered in the inner zone of the plots and 6% were found more than 15 cm away from the faeces in the outer zone of the plots: This proportion did not differ significantly between altitudes, runs or weeks (Fisher Exact Test: $p>0.05$).

The best model (AIC= 3014) included totalTEMP as a non-linear additive component and five further variables (firstTEMP, totalRAIN, firstRAIN and firstHUMID) as linear components. Furthermore the best model also included location as a random-effect variable. The addition of further variables did not reduce the model's AIC by more than two units, thus this model was selected as the best and most parsimonious model. The effect of each variable on the amount of larvae retrieved from grass is summarized in Fig. 4. According to our model, development of *H. contortus* larvae is positively correlated with humidity and air temperature during the first week, the total amount of rainfall and the amount of *H. contortus* eggs in the deposited faeces. On the contrary, the amount of rainfall during the first week was negatively correlated with herbage larval count. Additionally, there was an interaction between the amount of rainfall and the air temperature during the first week. Finally, our model showed a non-monotonic relationship between larval development and the sum of average daily temperatures with a peak around 450°C corresponding to four weeks with an average temperature of 16°C or three weeks with an average temperature of 21°C.

DISCUSSION

In this study, we report the successful development of infective larvae of *H. contortus* at altitude as high as 2000 above sea level in a temperate climate from the month of May to October. This corresponds to the altitudes and period of the year at which sheep are commonly grazed in Switzerland. The median number of larvae recovered per million deposited eggs on the fourth week was 262 and correspond approximatively to a 0.12% recovery rate. This is comparable with values between 0.02 and 1.5% reported in previous studies [8, 21]. Moreover, the proportion of larvae recovered at a distance more than 15 cm from the faeces was consistently low throughout all the trials, confirming previous observations [22].

There are several different processes are involved in the development of eggs to infective larvae on pasture,: hatching of eggs into first-stage larvae, migration of larvae outside from faeces to the soil and then to the grass, development of first-stage larvae to second and third-stage larvae as well as survival rate of eggs and larvae [3]. In the present study, it was not possible for practical reasons to assess all those different processes, and we used the amount of infective larvae recovered on the grass as a summary outcome for the development of *H. contortus* larvae.

Our model indicates a non-monotonic relationship between the total cumulated daily temperatures and the number of larvae present on the pasture, with a peak in larvae recovery at approximatively 450 cumulated degrees and a decrease in herbage larval count afterwards. Previous authors have reported the possible limiting effect of high temperature on larvae development: temperatures above 33-37°C are considered unfavourable to either the development of *H. contortus* or its ability to survive in the environment [2, 15, 23]. In our experiment, the peak of 450°C was reached in three or four weeks which correspond to an average temperature of 21°C or 16°C respectively, which lies far below this threshold. However, a total above 450°C cumulated degrees in 21-28 days under temperate climate conditions implies a series of days with intense sunshine and few clouds. Under such weather conditions, UV-light exposure is also expected to be detrimental to infective larvae development and survival. Finally, in our experimental trials, soil temperatures above 40°C were recorded several times, and although, infective larvae were still recovered afterwards, it is possible that the combination of high soil temperature and intense UV-exposure on hot summer days negatively affected the development of *H. contortus*. Moreover, although our results show an increase of larvae development over time, this predictive variable was not included in the best model. A possible explanation might be that the variable summarizing the total cumulated temperature and rainfall included in the model also contain a time component, making a further time variable such a week number redundant.

Additionally, our model indicates that conditions during the days immediately after the deposition of faeces are crucial for the parasite development. High humidity and warm temperature during the first week are associated with higher herbage larval count. However, heavy rainfall combined with cold temperature during the first week of the experiment reduced the quantity of retrieved larvae even though total rainfall is positively correlated with larval development. Several authors [24–26] reported a positive effect of rain on larval development. Nevertheless, Ndamukong et al., [27] observed a reduction in larval development in the case of heavy rainfall within the first nine days after faeces deposition and hypothesized that faeces were washed away by the rain before the hatching of the eggs and the migration of larvae outside the faeces occurs.

Similarly, larval herbage counts were negatively associated with cold temperatures during the first week after faeces deposition but there was no influence of temperature on the week prior to herbage larval count. This is consistent with results from Troell et al. [28], who reported a very low development rate of *H- contortus* from egg to infective larvae under daily fluctuation in temperatures between -1 and 15°C but a much higher survival rate of infective larvae under the same conditions. Those results highlight the ability of the parasite to quickly develop when the environment is favourable but also to endure under less than optimal conditions. This, combined with the parasite overwintering strategies inside the host [29] confirms the potential of the parasite to further expand its range in northern Europe as the climate there tends to become warmer [5, 9].

For the development of our model, we also considered including other temperature variables such as mean soil temperature, average of daily minimal and maximal air temperatures during the week, which might explain the observed variation in larval development better than mean air temperature [3, 23]. However, those variables were strongly correlated with mean air temperature (Pearson correlation coefficient>0.9). Hence, we choose to use only one variable to avoid collinearity issues. We gave preference to mean air temperature which is a commonly measured meteorological variable and will make our results easier to relate to other studies or to apply for management purpose. Gehrig-Fasela et al. [30] investigated the usefulness of using soil temperature rather than air temperature to model tree line limit in the Swiss Alps and coupling it to a soil-to-air temperature transfer model in order to take advantage of the availability of air temperature data for transposing their model to other areas. They concluded that although the coupled models performed slightly better than a model based on air temperature only, the costs in time and computing power of the coupled models made its usefulness debatable.

In conclusion, the work presented here has important implications regarding parasite control and herd management. A better knowledge of the ecology of *H. contortus* will help implement targeted strategies against the parasite such as pasture rotation, evasive grazing management or anthelmintic treatment timed to correspond to the period where animals on pastures are the most at risk. This is of particular significance in the face of the increasing anthelmintic resistance

[31] and the predicted change in the geographical and seasonal occurrence of the parasite due to global warming [5, 9].

To our knowledge, this study is the first to investigate thoroughly the ecology of the parasite *H. contortus* outside its hosts in temperate regions using a robust statistical model in order to evaluate the combined effect of several key parameters on the parasite's ability to develop on pastures. Although the use of standard meteorological indicators might reduce the precision of the model in comparison to more specific parameters such as soil temperature or humidity content of the faeces, those indicators are easily retrieved or derived from meteorological data around the world, which would enable practical application of our results to predictive modelling and management purpose.

Competing interests

The authors declare that they have no competing interests.

Authors' contribution

PT and HH participated in the design of the study. FM and JS conducted the experimental trials. FM carried out the analysis under PT and HH guidance and drafted and finalized the manuscript and all authors contributed to and approved the final version.

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Chapter III - Tables and Figures

Table 1: Summary of predictive variables included in a generalized additive mixed model to explain the amount of infective larvae of *Haemonchus contortus* retrieved by herbage larval count after deposition of 50 grams of infected sheep faeces.

Variable name	Description	Effect	Investigated relationship
prevTEMP	Average air temperature during the week preceding herbage larval count	fixed	additive or linear
firstTEMP	Average air temperature during the first week of the experiment	fixed	additive or linear
totalTEMP	Sum of average daily temperatures since the beginning of the experiment	fixed	additive or linear
prevRAIN	Rainfall during the week preceding herbage larval count	fixed	additive or linear
firstRAIN	Rainfall during the first week of the experiment	fixed	additive or linear
totalRAIN	Total rainfall since the beginning of the experiment	fixed	additive or linear
prevHUMID	Average air humidity during the week preceding herbage larval count	fixed	Linear
firstHUMID	Average air humidity during the first week of the experiment	fixed	Linear
FEC	Faecal egg count in faeces deposited on the plot	fixed	Linear
WEEK	Week number	fixed	Factorial
LOC	Experiment location	random	Factorial

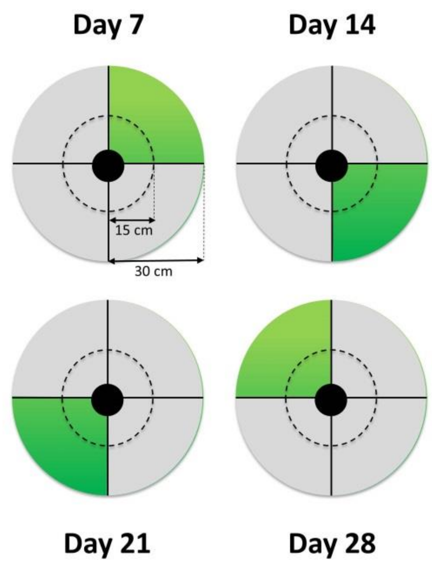

Figure 1: Schematic representation of the experimental plots. Faeces of sheep monoinfected with *Haemonchus contortus* was deposited in the centre of the plot on day 0 and grass was collected from one of four quadrants on days 7, 14, 21 and 28 for herbage larval count. Quadrants were collected following a clockwise rotation and are represented in green. Additionally, plots were separated in an inner (<15cm) and outer (>15cm) zone.

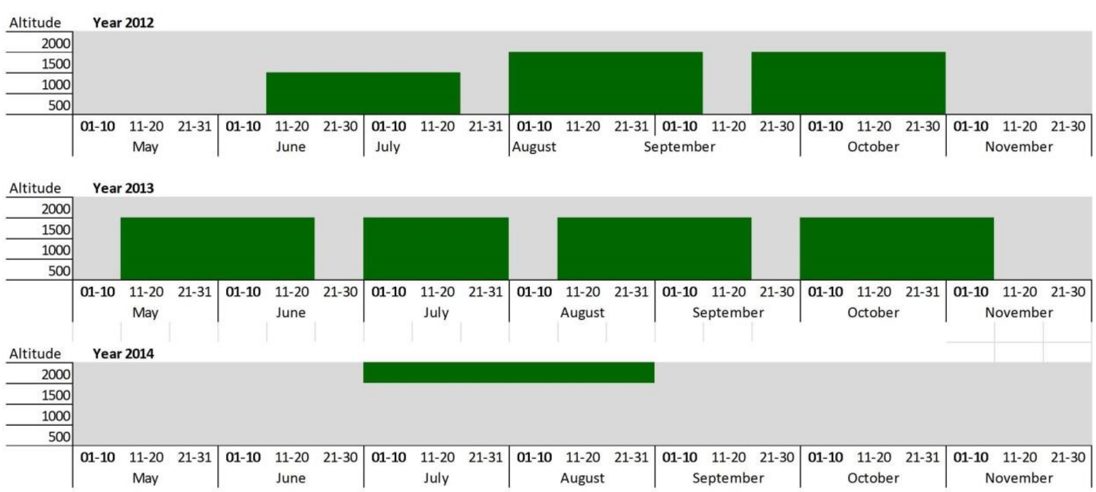

Figure 2: Timeline of the experimental trials (in green) assessing the development of *Haemonchus contortus* on alpine pasture situated at different altitudes.

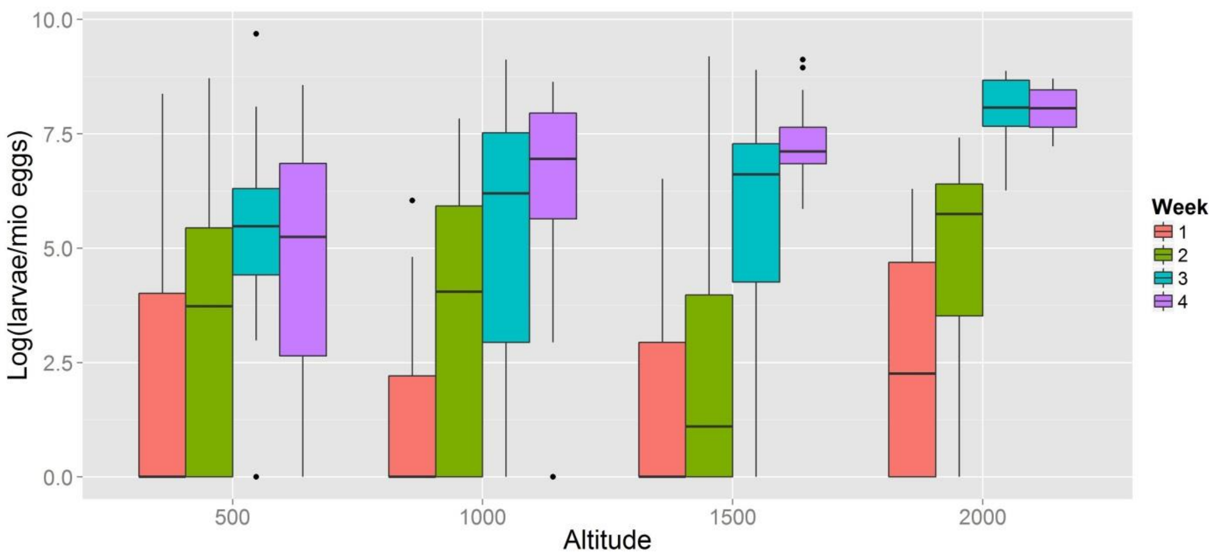

Figure 3: Boxplots showing the number of larvae of *Haemonchus contortus* recovered during weekly herbage larval count after deposition of 50 grams of infected sheep faeces on pastures at different altitudes. Results are grouped by altitudes (X-axis) and by weeks (different colours). Y-axis shows the log-transformed number of recovered larvae per million of eggs present in the deposited faeces.

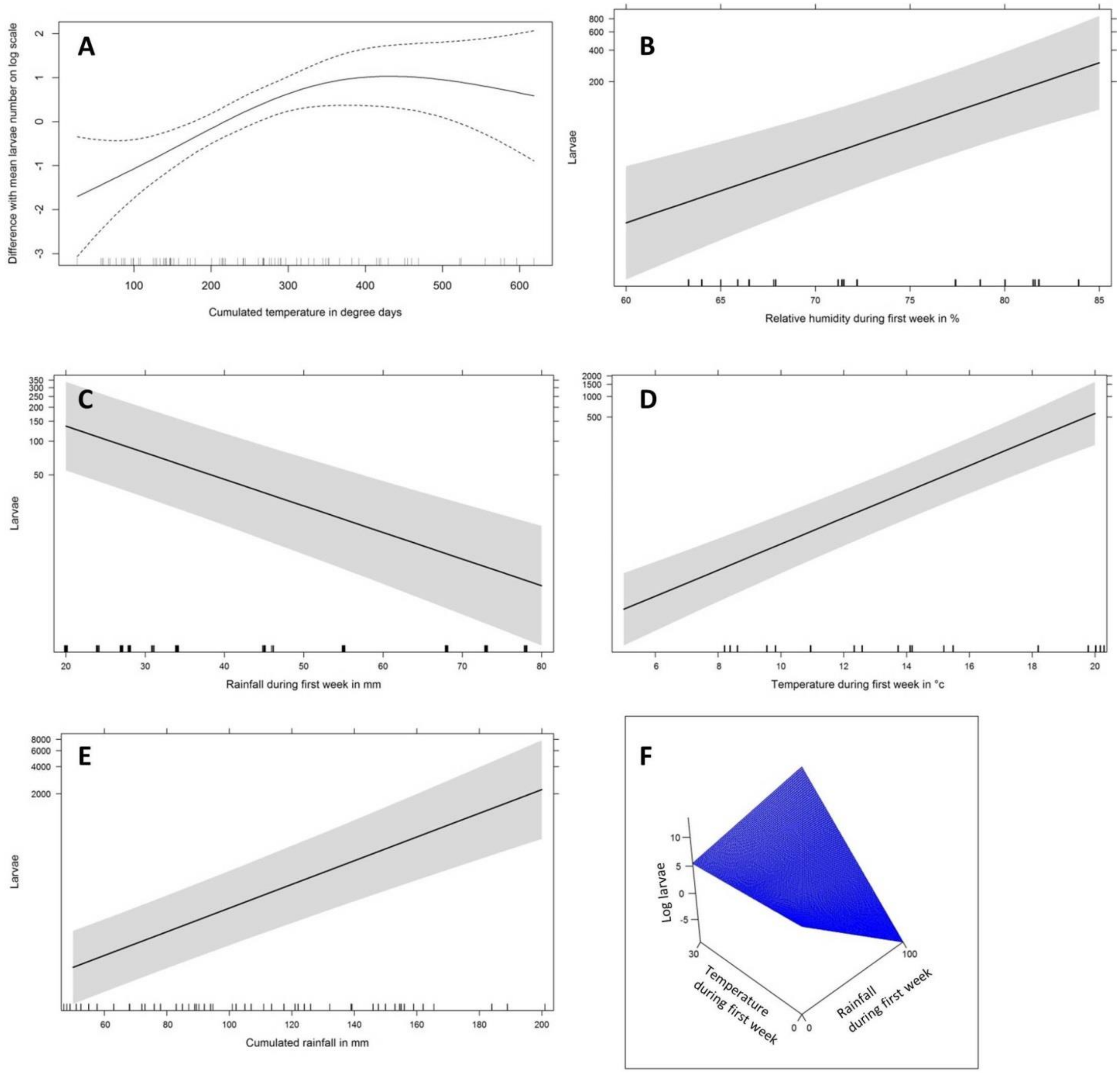

Figure 4: Estimated effect of the additive component (A: total cumulated average daily temperature) and the linear components (B-E: humidity during first week, rainfall during first week, temperature during first week and total cumulated rainfall) on the number of recovered *Haemonchus contortus* on pastures after deposition of 50 gram of infected sheep faeces and estimated with a general additive mixed-effect model. Dotted lines in panel A and grey areas in panels B-E represent the 95% confidence intervals. Panel F shows the interaction effect between rainfall during first week and temperature during first week.

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CONCLUSION

A large part of the work presented in this thesis has been the collection, review, and synthesis of the current available knowledge in order to assess the effect of nematode infection on sheep production and for the evaluation of the current situation in Europe regarding infection levels and financial losses due to nematode infection, deworming practises or anthelmintic resistance. This inventory work also served to put in relief topics where more research is warranted, namely:

- Impact of nematode on sheep wool and milk production: In the systematic review presented in the first chapter, I could retrieve some suitable studies focusing on those production traits, but they are far less in number when compared to the amount of studies dealing with weight gain in lambs. This precluded me from modelling losses in sheep wool and milk industry as I did for lamb meat. Although meat production is the main purpose of European sheep industry, wool and milk still represent an important source of income in some regions of the continent (wool mainly in United Kingdom, Spain, and Romania and milk in the Mediterranean region [1,2]).
- Beef cattle: In the second chapter, I could identify and take advantage of milk antibody levels and faecal egg counts as suitable indicators of nematode infection in dairy cows and meat sheep respectively: both those indicators have the double advantage of a) having a relationship with production losses that that has been described either in the present thesis or in previous work [3,4]; and b) being commonly reported in studies conducted in the different European countries. For beef cattle, although some studies described the effect of helminth infection on weight gain in beef cattle [5], they reported mainly the effect of anthelmintic treatment on cattle rather than the relationship between infection level and growth. Nevertheless, neither data on infection level or anthelmintic use in beef cattle are reported with sufficient details in Europe to allow a reliable assessment of the current situation concerning nematode infection and associated losses in this production type.
- Data from Eastern Europe: When modelling the nematode infection levels in Europe, I grouped countries in bio-economic regions in order to cope with missing data. Nevertheless, there was still an important lack of suitable data in Eastern European countries, to the point that several countries could not be included in the analysis (i.e.

Albania, Cyprus, Montenegro and Hungary). Additionally, even in Eastern European countries where data were available, the low number of available studies resulted in wide uncertainty intervals for our estimates. It is unclear whether this absence of data was due only to a lack of monitoring in those countries or if other factors such as language barrier or insufficient accessibility were also involved.

- Data on deworming practices: In the second chapter of the thesis, I summarized deworming practices in different European countries, reporting the percentage of farmers using anthelmintic drugs. Although the results give a useful insight on the main trends in both cattle and sheep, the lack of information on the type of drugs administered and their frequencies of use makes it difficult to provide a reliable assessment of the costs of deworming practices in European livestock.
- Cost-benefit analysis and productivity assessment: The results presented in chapter I and II focus on production losses rather than productivity. Several authors [6–9] stressed out the importance of integrating losses due to parasite into comprehensive cost-benefit model. This approach has the advantage to put in relation the direct losses due to a disease to other management factors (feeding, vaccine, treatment etc.). This allows expressing the impact of a disease as a measure of productivity that is the loss in output for equivalent input [10]. However, this approach requires an extensive knowledge of many important management parameters in the investigated farms and although recent cost-benefit analysis focusing on nematode infection in pigs or dairy cattle have been conducted at a national level in Europe [11,12], there is still insufficient data to provide continent-wide estimate of productivity losses due to gastro-intestinal nematode infection.

In spite of those limitations, the synthesis work that I conducted fills important gaps in knowledge concerning the impact of nematodes on sheep production and the current situation in Europe concerning nematode infection in dairy cattle and meat sheep.

With a total annual loss of over €1.2 billion for dairy cattle and meat sheep, the obtained estimates underline the importance of gastro-intestinal nematode infection for livestock industry in Europe. At the national level, those results are in line with figures reported previously [13,14]. For grazing dairy cattle only, the annual losses ranged between €22 and €83 per animal depending on the country. In comparison, worldwide estimates for losses due to clinical mastitis per cow and year range from €61 to €97 [15]. However, our model also highlights the importance of management factors such as age at slaughter or grazing practices in the final impact of nematode infection. This is in agreement with Perry et al. [7], who suggested that financial losses due to a disease should not alone be decisive for setting priorities and allocating resources but rather be considered in a larger socio-economic context.

The results presented in the second chapter, show that a non-negligible proportion of farmers using anthelmintic drugs (more than 90% for sheep and around 40% for dairy cattle). Those figures, together with the fact that 15 European countries reported anthelmintic resistance [16], highlight the potentially disastrous consequences for livestock production of the continuous use of anthelmintics as main worm control option. In particular for sheep, where anthelmintic resistance is becoming increasingly common and is considered to be directly responsible for substantial financial losses [17–19], alternative control options are more than ever needed. In addition the results from the harmonized coprological surveys conducted in Ireland, Italy and Switzerland showed large differences in prevalence for the helminths *Haemonchus contortus* and *Fasciola hepatica* [20,21]. Variation in prevalence followed a gradient along a north-south axis and was related to climatic factors such as temperature and rainfall, bringing further evidence of the potential for global warming to significantly modify the epidemiology of helminth infections in Europe.

This context underlines the importance of the experimental work on the development of *H. contortus* reported in the third chapter of the thesis. Importantly, the use of generalized mixed additive models allows assessing the combined effect of temperature, humidity and rainfall on the population dynamic of the free-living stage of the parasite. This approach allows describing the non-monotonic relationship between *H. contortus* development and the sum of daily air temperature expressed in degree-days which is a useful variable to describe cumulative biological processes [22,23]. Additionally, our results hint at the importance of weather conditions immediately after faeces deposition on pasture. The knowledge gained through this experimental study is complementary with other work on the development of helminth parasite conducted in the frame of the GLOWORM project [24–26] and will serve to improve already existing models aiming at predicting the consequences of climate change on helminth infections in Europe and developing sustainable alternatives to anthelmintic treatment.

In conclusion, helminth infection, its impact on livestock, the subsequent consequences on production and economy, as well as global warming and anthelmintic resistance are entwined together and form an extremely complex picture which requires a global vision in order to be fully appreciated. The work presented in this thesis inscribes itself in a collective effort from different research groups to take some steps toward this vision and to contribute to the efficient adaptation of helminth management in the face of the future challenges awaiting sheep and cattle industry.

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APPENDIX

- **Sheep and *Fasciola hepatica* in Europe: the GLOWORM experience (Geospatial Health, vol 9, 2015)**

- ***Haemonchus contortus*: spatial risk distribution for infection in sheep in Europe (Geospatial Health, vol 9, 2015)**

- **Widespread anthelmintic resistance in European farmed ruminants: a systematic review (Veterinary Records, 2015)**

Geospatial Health 9(2), 2015, pp. 309-317

Sheep and *Fasciola hepatica* in Europe: the GLOWORM experience

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Abstract. *Fasciola hepatica* infection challenges health, welfare and productivity of small ruminants throughout the world. The distribution of *F. hepatica* in sheep in Europe is usually scattered and studies are generally concerned with a single area making it difficult to compare results from different environments, climates and management regimes. In order to elucidate the current scenario in terms of prevalence and intensity of *F. hepatica* infection in sheep farms across Europe, a standardized cross-sectional survey was conducted in three pilot areas in Ireland, Switzerland and Italy, all part of the EU funded GLOWORM project. Two consecutive field surveys (in 2012 and 2013) were conducted in the three countries in the same period (August-October) in 361 sheep farms in total. Harmonized procedures (from farm to laboratory) based on pooled samples and the highly sensitive and accurate, diagnostic FLOTAC technique were used. The georeferenced parasitological results were modelled (at the pilot area level) following a Bayesian geostatistical approach with correction for preferential sampling and accounting for climatic and environmental covariates. The observed *F. hepatica* prevalence rates did not differ between the two study years in any of the three pilot areas, but they did vary between the countries showing high values in Ireland (61.6%) compared to Italy (7.9%) and Switzerland (4.0%). Spatial patterns of *F. hepatica* distribution were detected by the Bayesian geostatistical approach in Ireland with a high risk of infection in the south-western part of the pilot area there. The latent factor analysis highlighted the importance of year-to-year variation of mean temperature, rainfall and seasonality within a country, while long-term trends of temperature and rainfall dominated between countries with respect to prevalence of infection.

Keywords: *Fasciola hepatica*, sheep, geographical information systems, Bayesian modelling, Europe.

Introduction

Sheep farming has a prominent role in the sustainability of rural communities around the world (Park and Haenlein, 2006), as well as being socially, economically and politically highly significant at national and international levels, like all livestock species (Morgan et al., 2013). In the European Union (EU), there are currently around 98 million sheep (FAOSTAT, 2012). Efficient sheep livestock production is crucial to meet the increasing demands of meat and dairy products, especially in areas in which land is not arable (Chiotti and Johnston, 1995). However, several factors affect the productivity of the ovine sector in the EU, such as the capacity to maintain and improve farms (i.e. its health and genetic potential) and also the effect on human nutrition, community development and cultural issues related to the use of these livestock species (Nonhebel and Kastner, 2011). In many sheep rearing-countries, the emergence of a number of sheep parasitic infections, and inability to control them, has been reported in recent years (Taylor, 2012). This may be a reflection of alterations in sheep management and husbandry systems, climatic and environmental changes, over-reliance on anti-parasitic drugs and selection for resistance, or a function of them all (Taylor, 2012).

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Fasciola hepatica is a “well-known, old parasite of sheep” (Rojo-Vazquez et al., 2012), which continues to be a serious challenge to the health, welfare, productivity and reproduction of livestock throughout the world (Charlier et al., 2014). Due to its persistence and zoonotic role, more attention has been paid in the last few years towards this particular liver fluke. Aspects of the biology, epidemiology, diagnosis and control of *F. hepatica* infection in sheep have recently been reviewed (Rojo-Vazquez et al., 2012; Taylor, 2012).

Moreover, new reports on the re-emergence of fasciolosis in sheep, which are likely due to climatic changes and/or environmental modifications, suggest that the epidemiological patterns are changing with increasing prevalence in both northern (Kenyon et al., 2009; Novobilský et al., 2014) and southern European countries (Martínez-Valladares et al., 2013; Bosco et al., 2015). However, field studies on the distribution of *F. hepatica* in sheep are usually scattered and concerned with a single area (province, region or country). Therefore, difficulties have arisen when it comes to comparing results from different study areas, climates and management regimes, or when dealing with results derived from different surveys performed with different sampling and diagnostic procedures.

The present study is part of the EU funded GLOWORM project (http://www.gloworm.eu/) with a focus on the importance of sheep farming and the variability of climatic, environmental and ecological conditions. The aim was to elucidate the prevalence and intensity of *F. hepatica* infection in sheep farms across Europe by a standardized, cross-sectional survey conducted in 2012-2013 in three pilot areas of Ireland, Switzerland and Italy.

Materials and methods

Study area

Cross-sectional surveys were conducted in pilot areas in three key European countries (Fig. 1): the Sligo and Leitrim Counties in Ireland (3,427 km^2), the cantons Zürich, Aargau, Thurgau and St. Gallen in Switzerland (6,044 km^2) and the Campania region in Italy (13,598 km^2). The total number of sheep farms registered in each country and in each pilot area (both in 2012 and 2013) is shown in Table 1. In the three pilot areas small ruminant farming has a prominent role for the economy.

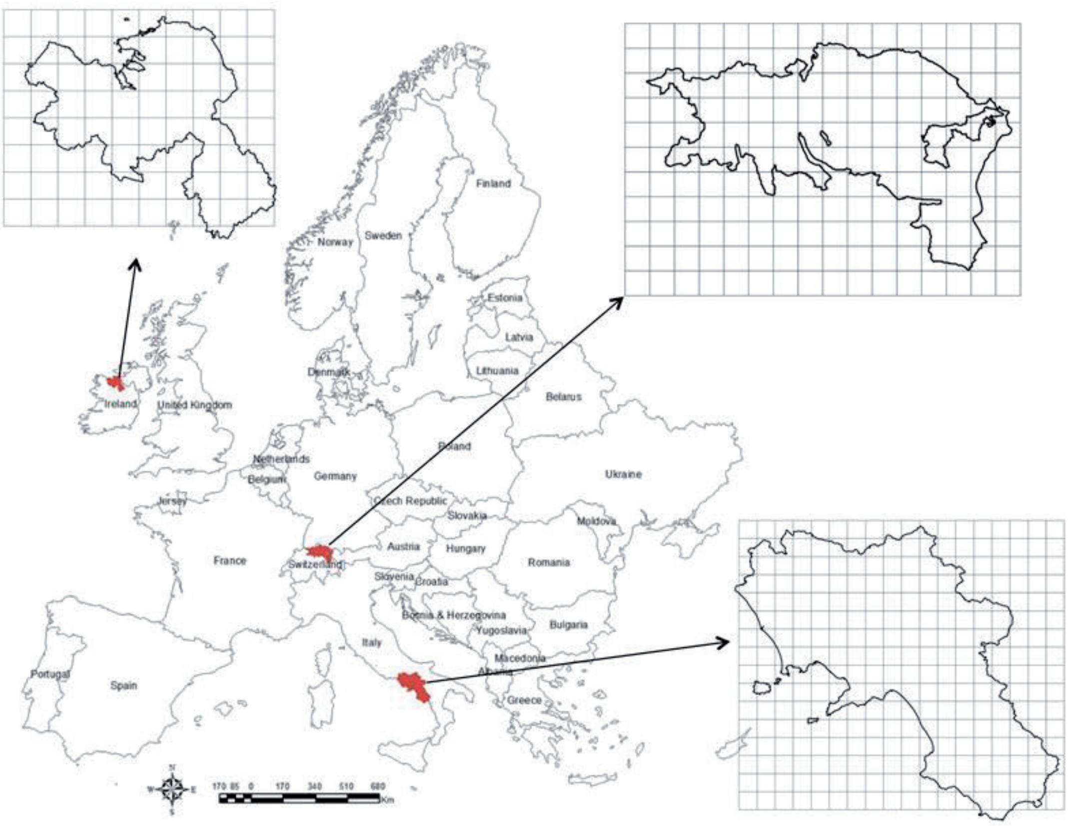

Fig. 1. Pilot areas (divided into 10 × 10 km quadrants) of Ireland, Switzerland and Italy where two consecutive, cross-sectional surveys were conducted in 2012 and 2013 within the GLOWORM project.

Study design, geographical information systems and farm sampling

Two consecutive field surveys (in 2012 and 2013) were conducted in the three countries in the same periods, i.e. during the second half of the sheep grazing season (August-October). A geographical information system (GIS), using the coordinate reference system ETRS 1989 Lambert Azimuthal Equal Area-LAEA, was developed using Arc-GIS 10.2.2 software (ESRI, Redlands, CA, USA). This was used, as reported by Musella et al. (2014), to construct a grid representing 10 × 10 km quadrants that could be overlaid on each pilot area map within the GIS as shown in Fig. 1. As a result, the pilot areas in Ireland, Switzerland and Italy were divided into 57, 95 and 135 equal quadrants, respectively (Fig. 1). All the farms sampled (each coded by a specific ID) were georeferenced using digital aerial photos (Google Earth) of the pilot areas (Rinaldi et al., 2006) and knowledge of the location of farms (pastures) by veterinarians and/or agricultural advisors working in each pilot area. We designed the same uniform survey with exclusion criterion of farms with less than 20 animals and inclusion criterion of being registered in local veterinary systems. In Ireland and Italy the farms were selected from the veterinary system database and invited to participate in the GLOWORM surveys. In the case of Switzerland they were provided by the Extension and Health Service for Small Ruminants. However, the predefined farm numbers could not be met due to the non-participation of some farms in each pilot area (around 15% in Ireland and 10% in both in Switzerland and Italy). It should be noted that, in the Campania region of southern Italy, previous surveys were used to evaluate how biased the survey sampling could be versus a systematic grid design (Musella et al., 2011, 2014). While properly conducted systematic grid sampling is straightforward in Italy, barriers to this approach exist in Ireland and Switzerland due to confidentiality and passive surveillance limitations. In the latter case, we were forced to carry out preferential sampling. In both cases, Bayesian geostatistical models taking informative/preferential sampling into account were developed (Catelan et al., 2015). A total of 361 sheep farms were tested in the three pilot areas during the two sampling periods (Table 1).

Animal sampling and laboratory procedures

For each farm, faeces were collected from 15 adult (older than 18 months) and 5 young (4-18 months) sheep (when possible). Veterinarians or farmers in each pilot area were asked to collect the samples for the surveys and were provided with a copy of the trial protocol and materials for faeces collection plus transport. Questionnaires with standard questions regarding farm management and epidemiological data were also recorded (data not shown). Once at the laboratory in each country, the samples were vacuum-packed (Rinaldi et al., 2011) and couriered to the central laboratory in Italy where they were analysed using standardized procedures. Specifically, for each farm, faecal samples were added together into 4 pools of 5 individual samples (Cringoli et al., 2002; Musella et al., 2011; Rinaldi et al., 2014). Each pooled sample was prepared, using equal amounts (2 g or less) from each individual faecal sample (Rinaldi et al., 2014). However, the predefined pool numbers could not be met when farms had less than 20 animals available for sampling, e.g. when young animals were not sampled. Hence, as can be seen in Table 3, the total number of pools examined from the 361 sheep farms was 1,079 (327 pools from young and 752 from adult sheep). For each pooled sample, faecal egg counts (FEC) were performed using the FLOTAC dual technique (Cringoli et al., 2010; Rinaldi et al., 2012) having an analytic sensitivity of 6 eggs per gram of faeces (EPG). A zinc sulphate-based flotation solution ($ZnSO_4$ specific gravity = 1.350) was used to detect and count *F. hepatica* eggs (Fig. 2).

Table 1. Total number of sheep farms registered in each country and in each pilot area (2012 and 2013) of the GLOWORM project.

Country	Number of sheep farms in the country		Number of sheep farms in the pilot area and compared to the whole country (%)	
	2012	2013	2012	2013
Ireland[1]	34,048	34,304	2,419 (7.1%)	2,433 (7.1%)
Switzerland[2]	9,169	8,903	2,114 (23.1%)	2,028 (22.1%)
Italy[3]	95,569	94,055	6,322 (6.6%)	6,380 (6.8%)

Source: [1]Department of Agriculture Food and the Marine (DAFM), Ireland; [2]Swiss Federal Statistical Office, Switzerland; [3]National Data Bank of the Livestock Registry, Italy.

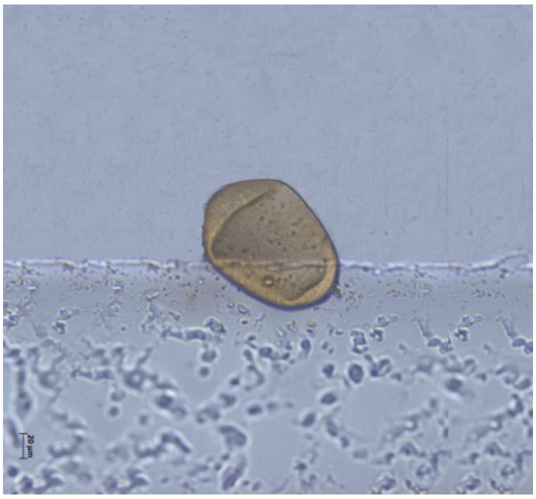

Fig. 2. Egg (slightly deformed due to flotation solution) of *F. hepatica* under FLOTAC (400x magnification).

GIS mapping, climatic and environmental variables

In order to display the distribution of *F. hepatica* in sheep farms located in the three pilot areas, point distribution maps were drawn (Fig. 3) within the GIS. In addition, the GIS for the study areas were implemented utilizing the climatic and environmental variables (Worldclim and MODIS datasets, http://www.worldclim.org/bioclim) as datalayers as described by Ducheyne et al. (2015). Elevation, slope and aspect of each pilot area were obtained from a digital elevation model (DEM) with a horizontal grid spacing of 30 arc-seconds (approximately 1 km) (source: GTOPO30, available from U.S. Geological Survey, EROS Data Center, Sioux Falls, South Dakota, USA). Data on each of these variables were then extracted and used as 3-km diameter "buffer zones" (Cringoli et al., 2002) centred on the georeferenced sheep farms (points).

Statistical methods

Parasitological data were summarized as proportion or averages and 95% confidence intervals (CI) calculated using standard approaches. Intensity of *F. hepatica* infection in each farm was assessed through the mean EPG calculated from positive pools. In addition, 25^{th}, 50^{th} and 75^{th} percentiles of *F. hepatica* EPG were also calculated.

Disease mapping was conducted specifying Bayesian geostatistical models based on WinBugs software (Lunn et al., 2000). However, the study design in the three areas was different from the standard approach due to the privacy restrictions mentioned above. Thus, there were two processes that had to be considered in the data analysis: the point process that governed the selection of farms and the

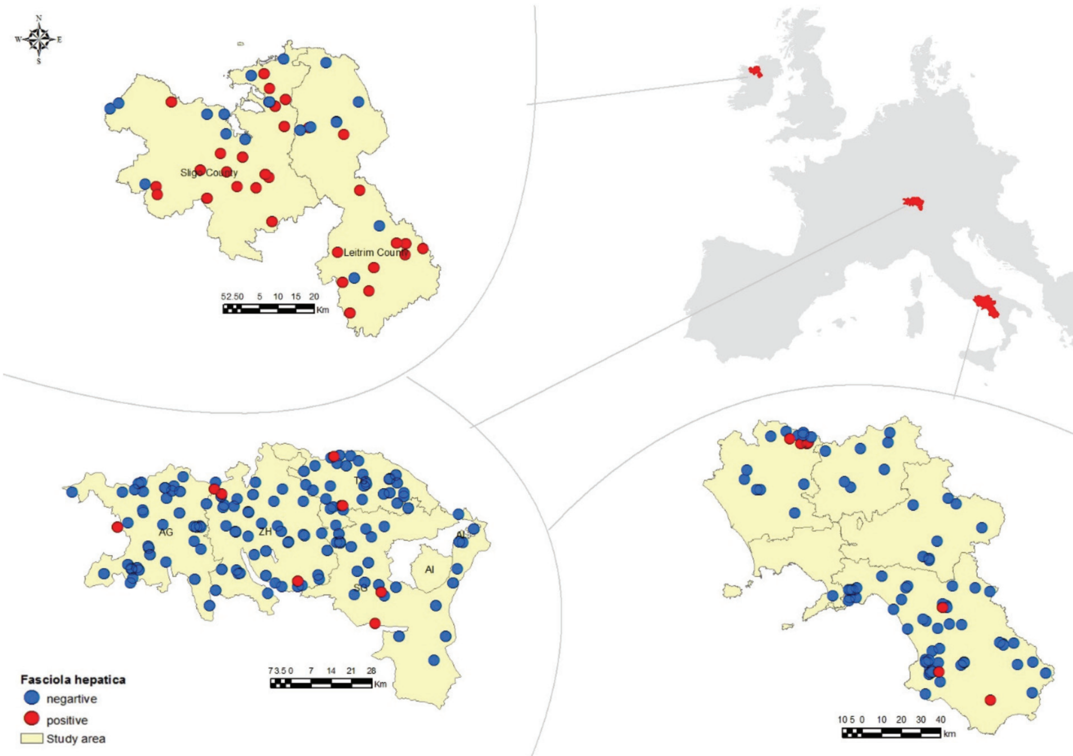

Fig. 3. Distribution of *F. hepatica* in sheep farms located in the pilot areas in Ireland, Switzerland and Italy - GLOWORM project 2012-2013.

Table 2. Prevalence and eggs per gram (EPG) of *F. hepatica* in sheep farms from the studied pilot regions within the GLOWORM project in the two study years (August-October 2012 and 2013).

Country	Number of sampled sheep farms			*Fasciola hepatica* in sheep farms in the pilot areas from three European countries					
				Number of positive farms (%) (95% Confidence Interval)			Mean EPG (25^{th} - 50^{th} - 75^{th} percentiles)		
	2012	2013	Total	2012	2013	Total	2012	2013	Total
Ireland	32	41	73	22 (68.8) (49.9 - 83.3)	23 (56.1) (39.9 - 71.2)	45 (61.6) (49.5 - 72.6)	18.7 (3.7 - 6.0 - 14.2)	25.6 (3.6 - 12.0 - 30-0)	22.2 (3.8 - 7.5 - 26.7)
Switzerland	73	126	199	4 (5.5) (1.8 - 14.2)	4 (3.2) (1.0 - 8.4)	8 (4.0) (1.9 - 8.1)	64.5 (2.5 - 8.2 - 183.0)	3.4 (1.9 - 3.0 - 5.2)	33.9 (1.9 - 3.7 - 10.5)
Italy	28	61	89	3 (10.7) (2.8 - 29.4)	4 (6.6) (2.1 - 16.8)	7 (7.9) (3.5 - 16.1)	25.6 (6.0 - 21.0 - 45.6)	30.2 (10.0 - 25.0 - 55.0)	27.8 (10.0 - 21.0 - 48.0)

continuous, spatial process for infection risk. Two separate, spatial random processes were specified. In step 1, a Bayesian analysis to model the spatial concentration of farm locations was performed (Diggle et al., 2010). We specified an inhomogeneous Poisson process with the count of sampled farms per grid cell and the total number of farms per grid cell as population denominator. From this model, posterior sampling probabilities per grid cell was obtained and used as weight in the geostatistical model fitted in step 2. Here, a Bayesian weighted geostatistical model with covariates on the presence/absence of *F. hepatica* infection was specified. A continuous risk surface of parasitic infection in the three study areas was then predicted using information from a large number of climatic and environmental variables obtained from MODIS and GIS data as described above. We avoided a selection step and preferred to reduce the dimensionality of the problem performing a Bayesian factor analysis, including three latent factors in the geostatistical model (Musella et al., 2011). A detailed description of the weighted Bayesian geostatistical model with latent factor analysis is described by Catelan et al. (2015).

Results

Observed field data

Fig. 3 shows the distribution of *F. hepatica* in sheep farms tested in the three pilot areas. The observed *F. hepatica* prevalence differed across the different European areas showing high values in Ireland (45/73 = 61.6%; 95% CI = 49.5-72.6%) compared to Italy (7/89 = 7.9%; 95% CI = 3.5-16.1%) and Switzerland (8/199 = 4.0%; 95% CI = 1.9-8.1%). The data showed a clustered spatial distribution of positive farms in Italy and Switzerland and a north-south gradient in Ireland. Table 2 shows the number of positive farms, the prevalence (number of positive farms over the total number of farms), including the 95% CI and the intensity of *F. hepatica* egg excretion (mean EPG calculated on the positive pools and the 25^{th}, 50^{th} and 75^{th} percentiles) by pilot area and study year.

The observed *F. hepatica* prevalence did not differ between the two study years in any of the three pilot areas. Table 3 shows the specific prevalence rates of *F. hepatica* by age group (adults and young sheep) related to pooled samples and controlled per-cluster

Table 3. Prevalence (95 % confidence interval) of *F. hepatica* in sheep by age (pooled samples) controlled for the cluster effect of pools within farms - GLOWORM project 2012-2013.

Age group	Number of pooled samples	Number of positive samples	Expressed as percent (95% CI)	Cluster-controlled outcome - expressed as percent (95% CI)
Adult	752	98	13.0 (10.7-15.7)	13.0 (10.0-17.0)
Young	315	12	3.8 (2.1-6.7)	3.7 (2.0-6.9)

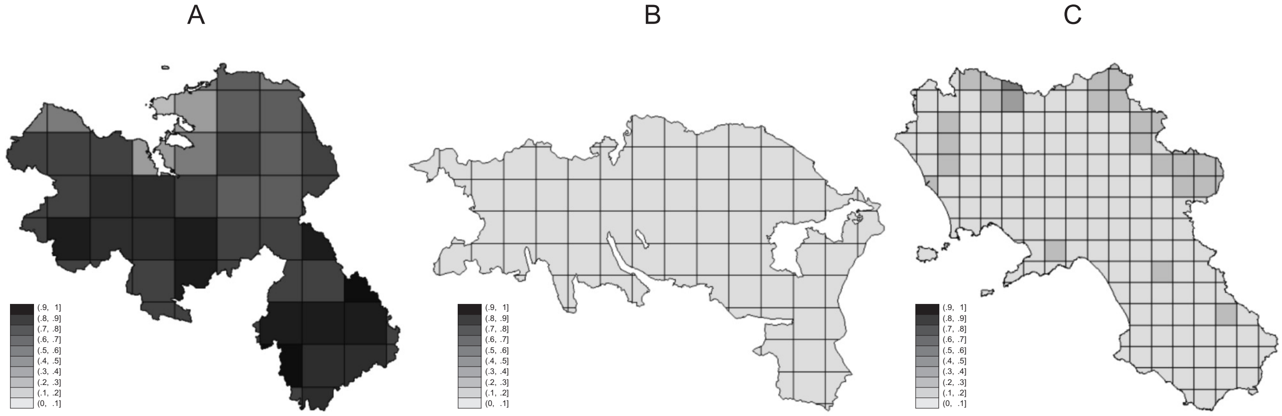

Fig. 4. Posterior predictive probability of *F. hepatica* infection in pilot areas in Ireland (A), Switzerland (B) and Italy (C) - GLOWORM project 2012-2013.

effect of pools within the farms. Liver fluke prevalence differed with respect to age being higher among older sheep *versus* younger ones (13 *versus* 4) respectively (Table 3). *F. hepatica* EPG in the positive pooled samples ranged from 6 to 558 EPG in adults (mean = 48.8; 25^{th}, 50^{th} and 75^{th} percentiles = 6.0, 12.0 and 43.5, respectively) and from 6 to 18 EPG in young sheep (mean 8.0; 25^{th}, 50^{th} and 75^{th} percentiles = 6.0, 6.0 and 10.5, respectively).

Bayesian geostatistical modelling

The georeferenced parasitological data from the 361 sheep farms in the pilot areas from Ireland, Switzerland and Italy were modelled according to a Bayesian geostatistical approach with correction for preferential sampling and accounting for GIS and remotely sensed covariates (Catelan et al., 2015). The latent factor analysis highlights the importance of year-to-year variation of the mean temperature, rainfall and seasonality within the country, while the long-term trend of mean temperature and rainfall dominated when the country-to-country prevalence rates were compared (Caminade et al., 2015; Ducheyne et al., 2015). The posterior predictive probabilities per grid cell for the pilot areas of the three investigated countries are shown in Fig. 4. The predicted prevalence of *F. hepatica* was higher in Ireland with a range from 11% to 81%. Fig. 4a shows that there is a spatial pattern in the distribution of the parasite in Ireland with a north-south gradient. *F. hepatica* was very rare in the pilot areas in Italy and Switzerland with a range of posterior predictive probabilities of 0.7%-30% and 1.4%-8.6%, respectively. In the pilot areas in Switzerland and Italy, the maps of the predicted probabilities were almost flat (Fig. 4 b,c).

To show the prediction uncertainty and the within-area variability, the posterior probability for each grid cell in excess with respect to the observed mean preva-

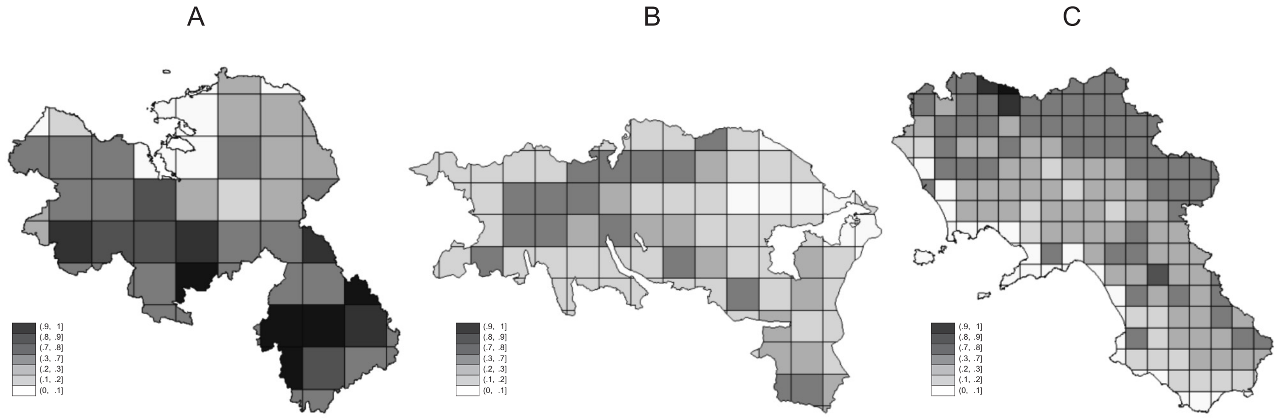

Fig. 5. Posterior probability to be in excess with respect to the average regional prevalence in pilot areas from Ireland (A), Switzerland (B) and Italy (C) - GLOWORM project 2012-2013.

lence can be seen in Fig. 5 (64% in Ireland, 4% in Switzerland and 8% in Italy).

The results confirm the high prevalence of *F. hepatica* in Ireland with higher risk of infection in the south-west part (Fig. 4). Despite of the overall low predicted prevalence in the pilot areas in Switzerland and Italy. it is possible to find a few grid cells with higher than average risk of infection (Fig. 5). However, the spatial pattern was weak, posterior probabilities being higher than average were below 70% in Switzerland and above 90% only in a small cluster of three grid cells in Italy.

Discussion

To our knowledge, this is the first harmonized, cross-sectional coprological survey on the prevalence and distribution of *F. hepatica* in sheep across an European north-south transect, conducted with the same methodology carried out in the same timeframe. As done for cattle by Ducheyne et al. (2015) for the GLOWORM project, we collected updated and reliable parasitological data for sheep through a standardized and harmonized approach based on cost-efficient, spatial sampling and diagnostic procedures involving pooled samples (Rinaldi et al., 2011) using the highly sensitive, accurate FLOTAC technique (Cringoli et al., 2010). This approach was used in order to: (i) construct high-quality maps to be disseminated to practitioners, farmers, decision-makers and other stakeholders in each pilot area/country; and (ii) use high-quality data as input for the Bayesian geostatistical models developed (Catelan et al., 2015).

The findings of the present study showed that the overall prevalence of *F. hepatica* in sheep farms in Europe is around 16%. However, results differed across the countries investigated showing high prevalence rates in Ireland (around 62%) and low rates in Switzerland (around 4%) and Italy (around 8%). These results for Italy are consistent with the low prevalence (1-12.4%) recently reported in sheep farms in southern Italy (Musella et al., 2011; Bosco et al., 2013). In addition, the severe outbreak of fasciolosis in sheep in southern Italy described in May 2014 by Bosco et al. (2015) indicates that changes in the epidemiology of *F. hepatica* at smaller scales could quickly occur as a consequence of climate change.

In the case of Ireland and Switzerland, only "personal communications" or "anecdotal" reports of fasciolosis in sheep exist, while systematic, cross-sectional surveys have not been performed to date. More attention has been paid in the last few years towards bovine fasciolosis in these two countries, with prevalence of 8.4-21.4% in Switzerland (Rapsch et al., 2008) and 65-82% (herds exposed to *F. hepatica*) in Ireland (Selemetas et al., 2015). In the case of Switzerland the differences between the *Fasciola* prevalences observed in sheep and cattle may be due to the different type of grassland offered to both species with consequences for the availability of the intermediate host.

The georeferenced results obtained during the harmonized surveys in the three countries were modelled (at pilot area level) following a Bayesian geostatistical approach with correction for preferential sampling and accounting for environmental covariates. Spatial patterns of *F. hepatica* distribution were clearly detected in Ireland with a high risk of infection in the south-western part of the pilot area. While the overall predicted prevalence was low, the spatial pattern of infection in the Campania region of southern Italy was similar to previous reports (Musella et al., 2011, 2014).

It is widely accepted that spatial distribution of *F. hepatica* is influenced by several contributing factors (Afshan et al., 2014), which are usually inter-dependent of each other. Some papers highlight climate factors as important predictors (Caminade et al., 2015), while others emphasize environmental features, such as vegetation indices (Valencia-López et al., 2012), and/or soil type (Selemetas et al., 2014), the presence of small water bodies (De Roeck et al., 2014), a combination of environmental factors (Musella et al., 2011, 2014), herd/flock density and other management factors (Bennema et al., 2011). The present study highlights the importance of year-to-year variation of mean temperature, rainfall and seasonality in the explanation of within-country frequency of infection, while the long-term trend of mean temperature and rainfall dominated country-to-country prevalence rates.

The present surveys emphasize that the *F. hepatica* prevalence rates differ in relation to age, the prevalence being higher in older animals that are exposed to a higher infection pressure by *F. hepatica* metacercariae due to longer periods at pasture. In the present paper, we highlight the need for integrating sound epidemiological designs with standardized diagnostic tools and strategies as well as geospatial tools for mapping helminth infections of sheep across Europe (Cringoli et al., 2013). Recognizing these challenges, harmonization of sampling and laboratory procedures, along with innovating, validating and applying new strategies, will foster and sustain long-term control of *F. hepatica* infections of livestock in Europe (Rinaldi and Cringoli, 2014).

In conclusion, the updated data and maps of the spatial distribution, prevalence rates of *F. hepatica* in European sheep has the potential to deliver improved directives with respect to the control of helminth infections for veterinarians, farmer associations and other stakeholders. The spatial sampling guidelines and the centralised GIS-based spatial data archive constructed during the GLOWORM project represents a guide for future epidemiological studies aimed at parasitological surveillance at different spatial scales.

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Haemonchus contortus: spatial risk distribution for infection in sheep in Europe

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Abstract. *Haemonchus contortus* is a species of gastrointestinal strongyles of primary concern for sheep. This highly pathogenic, blood-feeding helminth negatively influences animal health, welfare and productivity. In order to elucidate the current scenario in terms of prevalence and intensity of *H. contortus* infection in sheep farms across Europe, a standardized cross-sectional survey was conducted in three pilot areas in Ireland, Switzerland and Italy, all part of the EU funded GLOWORM project. Two consecutive field surveys (in 2012 and 2013) were conducted in the three countries in the same period (August-October) in 259 sheep farms in total. Harmonized, diagnostic procedures (from farm to laboratory) based on pooled samples, the FLOTAC technique and coproculture were used. The georeferenced parasitological results were modelled (at the pilot area level) following a Bayesian geostatistical approach with correction for preferential sampling and accounting for climatic and environmental covariates. The observed *H. contortus* prevalence rates did vary between the countries showing high values in Switzerland (77%) and Italy (73%) compared to Ireland (4%). Spatial patterns of *H. contortus* distribution were detected in Switzerland and Italy with a north-south gradient. The latent factor analysis highlighted the importance of seasonality and annual cyclicity within country (particularly in southern Italy), while mean temperature and rainfall dominated between country variations in the prevalence of *H. contortus* infection.

Keywords: *Haemonchus contortus*, gastrointestinal strongyles, spatial statistics, geographical information systems, sheep, Europe.

Introduction

Among parasitic infections of ruminants, gastrointestinal (GI) strongyles - caused by different genera of helminths (e.g. *Haemonchus*, *Ostertagia, Teladorsagia, Trichostrongylus, Cooperia, Oesophagostomum, Chabertia*) - continue to cause significant economic and welfare burden to the global livestock industry in Europe (Morgan et al., 2013; Charlier et al., 2014; Rinaldi and Cringoli, 2014). The ranking of these GI parasitic worms as one of the top causes of lost productivity in ruminants (http://www.discontools.eu) reinforces the increasing consideration in the European Union (EU) of the impact of strongyles upon animal health and productivity. However, these infections in grazing ruminants are often neglected with research and implementation of modern surveillance methods in this area remaining behind, mainly with regard to standardized diagnostic methods, surveillance and sustainable control strategies (Rinaldi and Cringoli, 2014).

Haemonchus contortus is a GI strongyle species of primary concern for small ruminants. It is a highly pathogenic, blood-feeding helminth that not only cause anaemia, but often also death in heavily infected animals (Burke et al., 2007). *H. contortus* has a very high propensity to develop resistance to anthelmintics (Kaplan et al., 2004) and drug resistance has been reported in farmed ruminants across Europe (reviewed in Rose et al., 2015). Geographically, it is widely distributed in tropical (between latitudes 23.5 N and

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23.5 S) as well as various subtropical climate zones (O'Connor et al., 2006). Prevailing climate (temperature, rainfall and moisture) and husbandry management practice are considered the main factors driving its spatial and temporal distribution.

Despite the parasite's strong association with tropical climates (Kao et al., 2000; O'Connor et al., 2006), its distribution range has recently expanded in northern temperate countries such as the UK where *H. contortus* in sheep is no longer rare (Kenyon et al., 2009; Burgess et al., 2012). Cases in Sweden (Hoglund et al., 2009) and Norway (Domke et al., 2013) have also been reported. Changes in climatic conditions have been implicated as the major driving force behind this expansion (van Dijk et al., 2008; Kenyon et al., 2009; Bolajoko et al., 2015). However, the spatial and temporal distribution of *H. contortus* is heterogeneous and depends on many different variables that vary from area to area, even from farm to farm (Musella et al., 2011). It is therefore important to monitor the prevalence and distribution of this helminth species to better plan sustainable control using targeted treatment and/or targeted selective treatment strategies (Cringoli et al., 2008; Kenyon et al., 2009).

In order to update the spatial distribution of risk of *H. contortus* infection in sheep farms across Europe, the aim of the present paper is to report the results of a standardized, cross-sectional survey conducted in the August-October period in 2012 and 2013 in three pilot areas of Ireland, Switzerland and Italy as part of the GLOWORM project, funded by the European Commission's (EC) seventh framework programme (FP7).

Materials and methods

The study area and sampling strategies have been described in a companion paper by Rinaldi et al. (2015). Briefly, two standardized coprological, cross-sectional surveys were conducted in 2012 and 2013 (August to October) on sheep farms (n = 361) located in pilot areas of three key European countries: Ireland (Sligo and Leitrim Counties), Switzerland (the cantons Zürich, Aargau, Thurgau and St. Gallen) and Italy (the Campania region).

Once at the laboratory of each country, the faecal samples were vacuum-packed and couriered to the central laboratory in Italy, where they were analysed using a harmonized diagnostic procedure that involved pooling samples (Rinaldi et al., 2014) and use of the FLOTAC dual technique (Cringoli et al., 2010; Rinaldi et al., 2011), with an analytic sensitivity of 6 eggs per gram (EPG) of faeces. A sodium chloride-based flotation solution (NaCl, specific gravity = 1.200) was used to detect and count the GI strongyles eggs as described by Rinaldi et al. (2011) (Fig. 1a). The total number of pooled samples examined from the 361 sheep farms was 1,079 (327 pools from young and 752 from adult sheep).

In order to identify *H. contortus* among the different GI strongyles present in mixed infections, a composite faecal culture (MAFF, 1986) was conducted for each farm. However, the predefined culture numbers (n = 361) could not be met for all farms since the amount of faeces was not always sufficient. The total number of farms tested by both FLOTAC and the coproculture approach was 259 (72% of the farms). *H. contortus* third-stage (*L3*) larvae were identified using the morphological keys proposed by van Wyk and Mayhew (2013) (Fig. 1b).

A geographical information system (GIS) was constructed utilizing the parasitological, climatic and environmental variables of the pilot areas in the three countries as data layers as reported by Rinaldi et al. (2015). The georeferenced parasitological data on *H. contortus* from the 259 sheep farms in the pilot areas from Ireland, Switzerland and Italy were used to construct a Bayesian geostatistical model with correction for preferential sampling and accounting for GIS and remotely sensed covariates (Catelan et al., 2015).

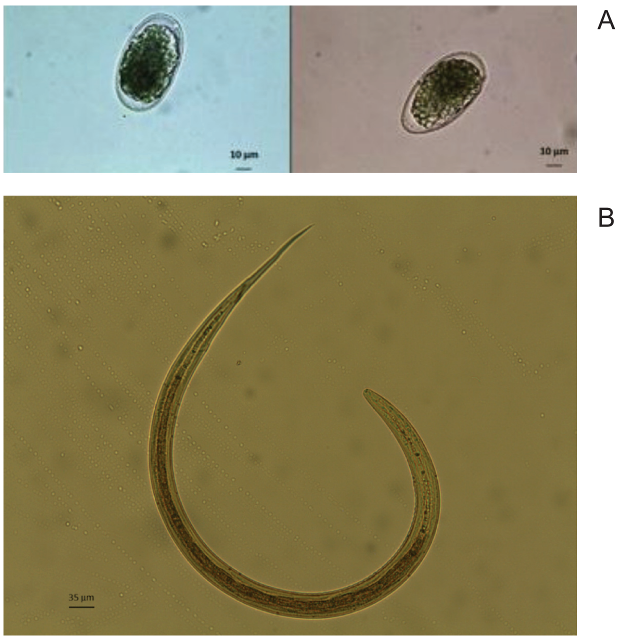

Fig. 1. Eggs of GI strongyles as they appear in the FLOTAC procedure (A) and third stage (*L3*) larva of *H. contortus* (B) (400X magnification).

Table 1. Prevalence and eggs per gram (EPG) of gastrointestinal strongyles in sheep farms from the studied pilot regions within the GLOWORM project in 2012-2013.

Country	Number of sampled sheep farms			Gastrointestinal strongyles in sheep farms in pilot areas from three European countries					
				Number of positive farms (%) (95% Confidence Interval)			Mean EPG (25^{th} - 50^{th} - 75^{th} percentiles)		
	2012	2013	Total	2012	2013	Total	2012	2013	Total
Ireland	32	41	73	32 (100.0%) (89.1-100.0%)*	41 (100.0%) (91.4 -100.0%)*	73 (100.0%) (95.1-100.0%)*	175.7 (50.6-120.1-236.5)	151.6 (59.4-92.0-165.9)	162.1 (56.0-100.8-206.7)
Switzerland	73	126	199	69 (94.5%) (85.8-98.2%)	113 (89.7%) (82.7-94.2%)	182 (91.5%) (86.4-94.8%)	606.7 (138.0-279.0-645.0)	586.8 (70.5-249.0-624.0)	594.2 (95.2-261.5-640.5)
Italy	28	61	89	28 (100.0%) (87.7-100.0%)*	57 (93.4%) (83.2-97.8%)	85 (95.5%) (88.2-98.5%)	461.8 (117.0-243.0-573.0)	562.3 (115.0-335.0-820.0)	529.2 (119.5-321.0-790.0)

*97.5% one-sided confidence interval.

Results

Observed field data

Table 1 reports, for each pilot area and for each study year, the number of farms positive for GI strongyles, the prevalence (number of positive farms over the total number of farms), 95% confidence intervals (CI), and the intensity of GI strongyle infection (mean EPG calculated on positive pools, including the 25^{th}, 50^{th} and 75^{th} percentiles). The overall prevalence of GI strongyles was very high across the three countries, i.e. 100.0% (97.5% one-sided CI = 95.1-100.0%) in Ireland, 91.5% (95% CI = 86.4-94.8%) in Switzerland and 95.5% (95% CI = 88.2-98.5%) in Italy.

Fig. 2 shows the distribution of *H. contortus* in sheep farms tested during the GLOWORM survey in the three pilot areas. The observed prevalence differed across the different European countries, showing high values in Switzerland (96/124 = 77.4%; 95% CI = 69.0-84.4%) and Italy (45/62 = 72.6%; 95% CI = 60.0-83.1%) with a very low prevalence in Ireland (3/73 = 4.1%; 95% CI = 0.9-11.5%). From the maps,

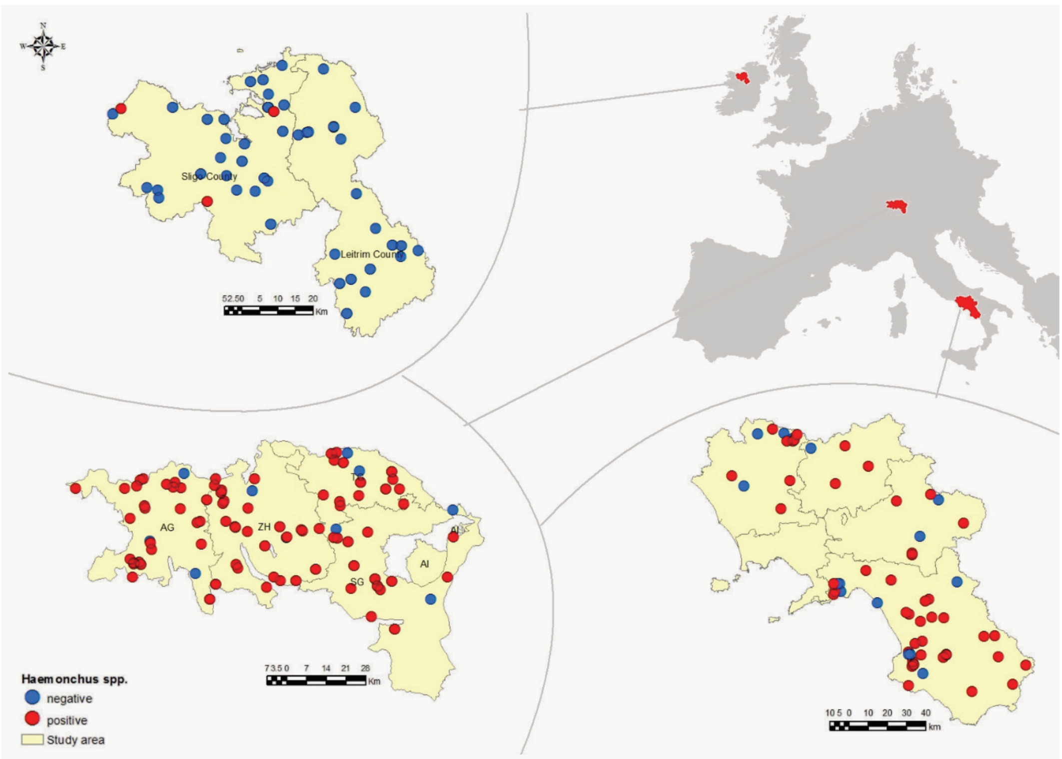

Fig. 2. Point distribution maps of *H. contortus* in sheep farms located in pilot areas of Ireland, Switzerland and Italy - GLOWORM project 2012-2013.

Table 2. Prevalence and eggs per gram (EPG) of *H. contortus* (deduced from the percentage of larvae) in sheep farms from the studied pilot regions within the GLOWORM project in 2012-2013.

Country	Number of sampled sheep farms			*Haemonchus contortus* in sheep farms in pilot areas from three European countries					
				Number of positive farms (%) (95% Confidence Interval)			Mean EPG (25^{th} - 50^{th} - 75^{th} percentiles)		
	2012	2013	Total	2012	2013	Total	2012	2013	Total
Ireland	32	41	73	1 (3.1%) (0.2-18.0%)	2 (4.9%) (0.8-17.8%)	3 (4.1%) (0.9-11.5%)	10.1 (.)	4.0 (1.4-3.9-.)	6.0 (1.4-6.5-.)
Switzerland	52	72	124	38 (73.1%) (58.7-84.0%)	58 (80.6%) (69.2-88.6%)	96 (77.4%) (69.0-84.4%)	152.7 (17.7-48.5-198.5)	462.3 (17.2-71.0-289.5)	339.8 (18.0-68.0-246.5)
Italy	7	55	62	4 (57.1%) (20.2-88.2%)	41 (74.5%) (60.7-84.9%)	45 (72.6%) (60.0-83.1%)	413.2 (3.7-13.0-1223.0)	193.0 (20.1-93.3-221.5)	212.2 (16.1-87.7-221.5)

it is difficult to recognize the within-country spatial pattern in the distribution of the positive farms.

Table 2 reports, for each pilot area and year, the number of farms positive to *H. contortus*, the prevalence (number of positive farms over the total number of farms) and 95% CI, and the intensity of *H. contortus* infection (mean EPG calculated on the positive pools, including the 25^{th}, 50^{th} and 75^{th} percentiles) deduced from the percentages of *H. contortus* larvae identified. The observed *H. contortus* prevalence did not differ between the two study years in any of the three pilot areas.

It should be noted that apart from *H. contortus* other GI strongyle genera (*Teladorsagia, Trichostrongylus, Cooperia, Oesophagostomum/Chabertia*) were also detected in the coprocultures with different prevalence values across the three countries (data not shown).

The georeferenced results were modelled using a Bayesian geostatistical approach with correction for preferential sampling and accounting for environmental covariates (Catelan et al., 2015). Latent factor analysis highlighted the importance of seasonality and annual cyclicity within the countries (particularly in the Campania region of southern Italy), while mean temperature and rainfall dominated between country variations in the prevalence of *H. contortus* infection. The posterior predictive probabilities per grid cell for the pilot areas of the three investigated countries are reported in Fig. 3. The predicted prevalence of *H. contortus* was higher in Italy and Switzerland with a range of posterior predicted probabilities of 26.8%-90.8% and 33.7%-91.5%, respectively. In Italy, it was possible to capture a weak spatial pattern with a north-south gradient in the distribution of the parasite (Fig. 3c). The spatial pattern was less clear in Switzerland. *H. contortus* was very rare in sheep in Ireland and the spatial pattern almost flat. For only one grid cell the posterior predictive probability of infection was higher than 80% (Fig. 3a).

To show the prediction uncertainty and the within-area variability, we report also the posterior probability for each grid cell to be in excess with respect to the observed mean prevalence (4.1% in Ireland, 77.4% in Switzerland and 72.6% in Italy), which is shown in Fig. 4.

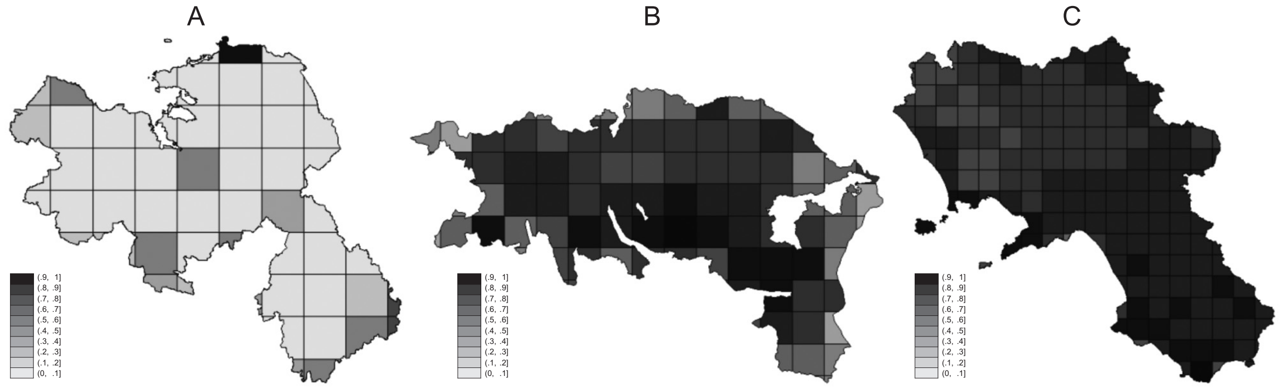

Fig. 3. Posterior predictive probability of *H. contortus* infection in pilot areas from Ireland (A), Switzerland (B) and Italy (C) - GLOWORM project 2012-2013.

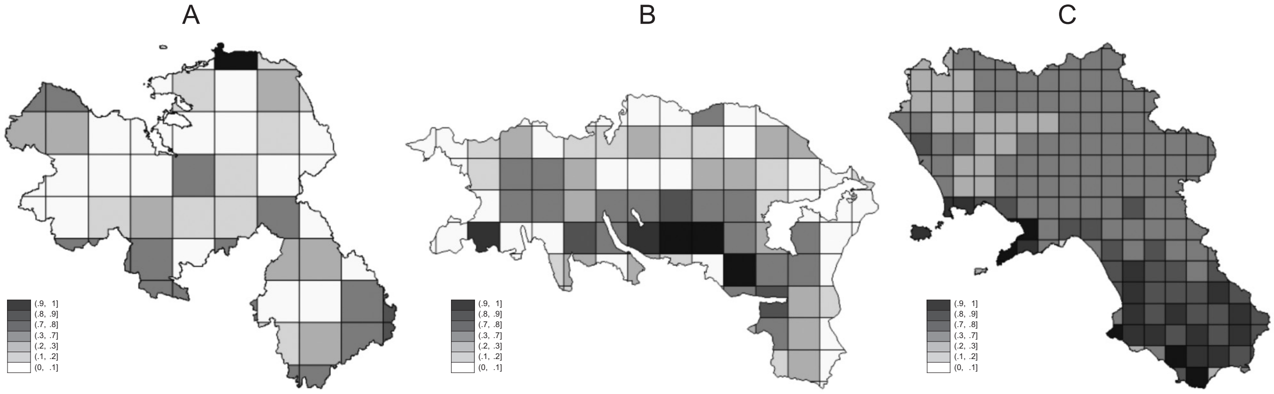

Fig. 4. Posterior probability in excess with respect to the average regional prevalence of *H. contortus* in pilot areas from Ireland (A), Switzerland (B) and Italy (C) - GLOWORM project 2012-2013.

The map of the Campania region of southern Italy confirms the spatial distribution of *H. contortus* reported in Fig. 3c, with the infection risk higher than the regional average on the coast and in the southern part of the region. We recognized a spatial pattern also in the pilot area of Switzerland with a south to north gradient (Fig. 3b). Despite the overall low predicted prevalence, it was possible to recognize one grid cell with higher than average risk of infection in Ireland. The spatial pattern was consistent with that of posterior predictive probabilities (Fig. 3a).

Discussion

Infections by GI strongyles are arguably the most important causes of suboptimal productivity in sheep (Nieuwhof and Bishop, 2005; Cringoli et al., 2008). During the GLOWORM project we collected updated and reliable data on these helminths through a standardized and harmonized approach based on cost-efficient, spatial sampling and diagnostic procedures involving pooled samples (Rinaldi et al., 2011) and the highly sensitive, accurate FLOTAC technique (Cringoli et al., 2010). As expected, a very high prevalence of GI strongyles was found in sheep farms in the pilot areas of the three investigated countries: 100.0% in Ireland, 91.5% in Switzerland and 95.5% in Italy.

Grazing sheep are frequently parasitized by multiple species of GI strongyles (mixed infections), and knowledge of the species of GI strongyle present in areas where sheep farming is relevant for the local economy, is important in order to plan control and treatment strategies. Among the GI strongyles of sheep, we focused on *H. contortus* in the present paper, due to its pathogenicity, ubiquity, high biotic potential, dependence on climatic/environmental factors and propensity to develop resistance to anthelmintics. Based on the results gathered from GLOWORM, the overall prevalence of *H. contortus* in sheep farms in Europe was around 56%. However, results differed across the countries investigated showing high prevalence rates in Switzerland (around 77%) and Italy (around 73%) and low rates in Ireland (around 4%).

The large number of prevalence surveys and studies of field epidemiology in diverse regions provided a picture of scattered *H. contortus* distribution in Europe so far. In the present paper, we report results from a first harmonized, cross-sectional, coprological survey on the prevalence and distribution of *H. contortus* in sheep across an European north-south transect, conducted with the same methodology carried out in the same timeframe.

In agreement with the model proposed by Bolajoko et al. (2015), our field data confirm that *H. contortus* exhibits spatial heterogeneity in its infection pressure based on different, prevailing climate zones, i.e. in Ireland, Switzerland and Italy. In line with the distribution in the southern hemisphere (Kao et al., 2000), *H. contortus* tends to be more common and a greater risk to sheep health and production in warmer areas in the South, such as those of southern Italy (Musella et al., 2011, 2014) and Switzerland. It should be noted that the farms sampled in Switzerland were only located in the midland area (300-1,000 m altitude), where clinical haemonchosis is of considerable higher importance compared with the cooler mountainous regions (H. Hertzberg, personal communication).

Difference in *H. contortus* prevalence in the different countries studied could also depend on the management practice and treatment regimes used by the farmers. As an example, *H. contortus* is the predominant, resistant species against benzimidazole and moxidectin in Switzerland (Meyer, 2001; Scheuerle et al., 2009). On the contrary, anthelmintic resistance is very rare in sheep in southern Italy, a region where anthelmintic use is limited (Cringoli et al., 2008; Rinaldi et al., 2014).

In the present study, in order to model the distribution of *H. contortus* in the various study areas, a Bayesian geostatistical model was developed with correction for preferential sampling and accounting for environmental covariates (Catelan et al., 2015). Spatial patterns of *H. contortus* distribution were detected in Switzerland and Italy with a north-south gradient in agreement with the findings reported by Musella et al. (2011) for the Campania region in southern Italy. The latent factor analysis highlighted the importance of seasonality and annual within-country cyclicity (particularly in the Campania region of southern Italy), while mean temperature and rainfall dominated in the variations of *H. contortus* infection from country to country. These results are in agreement with data from the literature; indeed the development and survival of free-living stages of *H. contortus* depend strongly on temperature and water availability; thus, transmission is strongly influenced by seasonal changes in the prevailing climate (Van Dijk et al., 2008; Morgan and Van Dijk, 2012). The susceptibility of *H. contortus* eggs and pre-infective stages (*L1* and *L2*) to desiccation (Rossanigo and Gruner, 1995) is highly characteristic of this helminth species, limiting its distribution to areas with warm, moist summers and creating a natural barrier to development that results in sporadic development of the free-living stages (O'Connor et al., 2007). In previous studies conducted in southern Italy (Musella et al., 2011, 2014), sparse vegetation and rivers, mixed and permeable soil explained the spatial distribution of *H. contortus* in sheep.

The output generated by GLOWORM provide a spatial database incorporating parasitology, farm management, environmental information and climatic data. This common and standardised database is useful to develop cost-efficient sustainable sampling strategies, and multi-scale spatial models for parasite occurrence, including the impact of climate change and anthelmintic resistance in livestock in Europe (Bolajoko et al., 2015; Rose et al., 2015).

Predictive models (based either on time series analysis or on basic reproduction rate model) of *H. contortus* transmission to sheep have recently been developed by Bolajoko et al. (2015). However, collection of prevalence data derived from active surveillance, as those from the present cross-sectional survey, are necessary for continued models improvement, validation and meaningful predictions (Fox et al., 2012; Bolajoko et al., 2015). Promoting standardized cross-sectional surveys among practitioners and farmers is one of the priority areas for an integrated sustainable control of *H. contortus* and other helminths in sheep.

Acknowledgements

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Short Communication

Widespread anthelmintic resistance in European farmed ruminants: a systematic review

H. Rose, L. Rinaldi, A. Bosco, F. Mavrot, T. de Waal, P. Skuce, J. Charlier, P. R. Torgerson, H. Hertzberg, G. Hendrickx, J. Vercruysse, E. R. Morgan

Anthelmintic resistance (AR) in gastrointestinal nematodes (GINs) has been reported worldwide in multiple nematode and livestock species (Kaplan and Vidyashankar 2012) and is a major constraint on production on affected farms (Sutherland and others 2010, Miller and others 2012). In the UK and Ireland, for example, AR in GINs and anthelmintic treatment failure is widespread in sheep (e.g. Bartley and others 2003, Keane and others 2014) and increasingly reported in cattle (e.g. O'Shaughnessy and others 2014). There is, therefore, a need to develop and adopt GIN control strategies that maintain the efficacy of anthelmintics and to identify risk factors for the development of AR.

Environmental constraints on farm management and the survival of nematodes in refugia appear to play an important role in the development of AR. In a random survey of sheep farms in Norway, AR was found only in coastal regions (Domke and others 2012a). Papadopoulos and others (2001) observed a higher incidence of AR on isolated Greek islands, suggesting that drought hastens the development of AR. In contrast, Rinaldi and others (2014) observed high anthelmintic efficacy in sheep in southern Italy despite the Mediterranean climate. This was attributed to the low number of anthelmintic treatments (usually two per year) and the absence of anthelmintic treatments during periods of drought, when environmental constraints on the free-living stages are highest. Calvete and others (2012) identified an association between AR, distance between farms with AR, management and bioclimatic variables on sheep farms in Aragon, Spain. In particular, the association between AR and climatic conditions was attributed to the application of anthelmintic treatments during the winter months, which increases the selection pressure on the already depleted population of nematodes in refugia. Such spatial analyses provide useful insights into risk factors for AR, but their application is likely to be limited outside of the region studied. Pan-European spatial analysis and modelling of the distribution of AR may enable the elucidation of common risk factors for the development of AR in European livestock.

A systematic review of peer-reviewed literature was undertaken to record the current distribution of AR in the major GINs (*Teladorsagia* species, *Trichostrongylus* species, *Haemonchus contortus*, *Ostertagia ostertagi* and *Cooperia oncophora*) infecting goats, sheep and cattle in Europe (defined as the EU, European Economic Area and Switzerland). The ISI Web of Science database was explored using the keywords "anthelmintic resistance" (last searched 2 Oct 14). No restrictions were placed on publication dates. The search yielded 1852 publications, of which 120 publications were selected based on title and abstract, excluding studies on non-ovine, non-bovine or non-caprine hosts and nematodes, non-European studies and studies where AR arose through artificial selection. A further nine reports of AR were identified from citations, MSc/PhD theses and authors' unpublished data. Of these publications, 73 provided reports of AR in cattle, sheep or goats assessed in accordance with the World Association for the Advancement of Veterinary Parasitology guidelines (Coles and others 1992) and stated the country or region where the farms were located.

AR in GINs, assessed primarily using faecal egg count reduction tests, is widespread in Europe (see online supplementary figure). Overall, AR was reported in all five GIN genera and in 16 countries throughout Europe (see online supplementary figure and table). Multiple drug resistance (MDR) in the three main GIN genera infecting sheep and goats was reported in 10 countries (see online supplementary table). Not all studies tested multiple anthelmintics, and therefore, MDR is likely to be more widespread. Monepantel resistance was reported on sheep farms in the Netherlands in November 2014 (Anon 2014) but was not included in the systematic review as details regarding the methods used to assess resistance were not available at the time of writing. AR against derquantel had not been reported in Europe at the time of writing. However, due to publication and sample selection bias, the absence of reports of AR in some regions may simply be due to a lack of monitoring and AR cannot be considered absent elsewhere. Heterogeneity in the distribution of AR in Europe might also depend on the lack of standardised procedures for surveys and detection of AR on farms and in laboratories.

The estimated prevalence of AR varied by region, anthelmintic class and host. Random surveys of sheep farms have detected albendazole resistance on 11 per cent of farms in Norway (n=19; Domke and others 2012a); ivermectin, benzimidazole and levamisole resistance on 23 per cent, 3.7 per cent and 7.4 per cent of farms, respectively, in Slovakia (n=27; Čerňanská and others 2006); and benzimidazole and levamisole resistance

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on 83 per cent and 50 per cent, respectively, of farms in western France (n=23; Chartier and others 1998). In the latter study, benzimidazole resistance was also detected on 93 per cent of goat farms (n=15). A further random survey of dairy goat farms in southwestern France detected benzimidazole resistance on 83 per cent of farms and multiple resistance to benzimidazole and levamisole on 11 per cent of farms (n=18; Chartier and others 2001). A sample size-weighted mean prevalence of benzimidazole resistance in GINs in sheep and goats of 50.1 per cent was estimated from the above four studies. Excluding goats, the sample size-weighted mean prevalence of benzimidazole resistance in sheep GINs was 32.1 per cent. Insufficient data were available to estimate mean prevalence for other anthelmintic classes and cattle. The prevalence of AR has also been estimated elsewhere, for example, treatment failure has been identified on 51 per cent of Irish sheep farms surveyed (Keane and others 2014) and 64 per cent of Scottish sheep farms surveyed (Bartley and others 2003). These studies provide valuable prevalence estimates, but the differences in sample (farm) selection methods introduce potential sample selection bias and may affect estimates and comparability between regions. For example, Domke and others (2012a) observed AR on 33 per cent of randomly selected sheep flocks and 80 per cent of non-randomly selected sheep flocks in the Rogaland region of Norway. Therefore, it is recommended that future prevalence surveys follow a random or stratified sampling approach where possible to reduce sample selection bias.

The biases described above currently prevent robust spatial meta-analysis of AR in Europe and restrict the spatial analysis that can be undertaken. In addition, since spatial analysis is rarely the purpose of a study into AR and due to data protection responsibilities, cases are usually reported at a country or regional level. Due to the significant within-region heterogeneity in the distribution of AR (e.g. Calvete and others 2012), data with a higher spatial resolution are required.

Taken together, the peer-reviewed literature paints a picture of widespread AR in Europe with the potential for high regional prevalence. Veterinarians should continue to promote sustainable anthelmintic use (e.g. Abbott and others 2012, Charlier and others 2014), even on farms where AR is not suspected. Continued surveillance of AR in Europe, reporting the absence of resistance (Paraud and others 2010, Rinaldi and others 2014) and reporting cases in a way that enables spatial meta-analysis, will aid in the future identification of risk factors and evaluation of sustainable nematode control practices.

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Widespread anthelmintic resistance in European farmed ruminants: a systematic review

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Supplementary table. Summary of anthelmintic resistance reported in five major gastrointestinal nematode species/genera infecting goats, sheep and cattle in Europe.

AH class[a]	Host	Region	References
***Teladorsagia* spp.**			
BZ	**Sheep**	Czech Republic, Denmark, France, Greece, Ireland, Italy, Netherlands, Norway, Slovakia, Spain, UK	Alvarez-Sánchez and others 2006, Bartley and others 2004, 2006, Bjørn and others 1991, Boersema and others 1987, Borgsteede and others 1997, 2007, Britt & Oakley 1986, Cawthorne and Whitehead 1983, Cawthorne and Cheong 1984, Čerňanská and others 2006, Chartier and others 1998, Díez-Baños and others 2008, Domke and others 2012a,b, Geurden and others 2014, Good and others 2012, Grimshaw and others 1994, Hong and others 1992, 1996, Maingi and others 1996b, Martínez-Valladares and others 2012a, McMahon and others 2013, Mitchell and others 2010, Taylor and others 2009, Traversa and others 2007, Vadlejch and others 2014
	Goats	France, Denmark, Italy, Netherlands, Norway, Spain, UK	Borgsteede and others 1996, Chartier and others 1998, 2001, Domke and others 2012a, Hong and others 1996, Jackson and others 1992, Maingi and others 1996a, Requejo-Fernández and others 1997, Zanzani and others 2014
ML	**Sheep**	Czech Republic, Denmark, Italy, Netherlands, Slovakia, Spain, Sweden[b], UK	Alvarez-Sánchez and others 2006, Bartley and others 2004, 2006, Borgsteede and others 1997, Čerňanská and others 2006, Díez-Baños and others 2008, Höglund and others 2009, Maingi and others 1996b, Martínez-Valladares and others 2012a, b, McMahon and others 2013, Taylor and others 2009, Traversa and others 2007, Vadlejch and others 2014
	Goats	Denmark, Italy, Switzerland, UK	Jackson and others 1992, Maingi and others 1996a, Murri and others 2014, Zanzani and others 2014
LEV	**Sheep**	Denmark, France, Greece, Ireland, Italy, Netherlands, Spain, UK	Alvarez-Sánchez and others 2006, Bartley and others 2004, 2006, Bjørn and others 1991, Borgsteede and others 1997, Chartier and others 1998, Geurden and others 2014, Good and others 2012, Hong and others 1996, Maingi and others 1996b, Martínez-Valladares and others 2012b, McMahon and others 2013, Mitchell and others 2010, Taylor and others 2009, Traversa and others 2007
	Goats	Denmark, France, UK	Chartier and others 2001, Hong and others 1996, Maingi and others 1996a

MDR	**Sheep**	Denmark, Greece, Ireland, Italy, Netherlands, Spain, UK	Alvarez-Sánchez and others 2006, Bartley and others 2004, 2006, Borgsteede and others 1997, Geurden and others 2014, Good and others 2012, Maingi and others 1996b, Martínez-Valladares and others 2012b, Mitchell and others 2010, Sargison and others 2005, 2007, 2010, Taylor and others 2009, Traversa and others 2007, Wilson & Sargison 2007
	Goats	Denmark, France, UK	Chartier and others 2001, Jackson and others 1992, Maingi and others 1996a

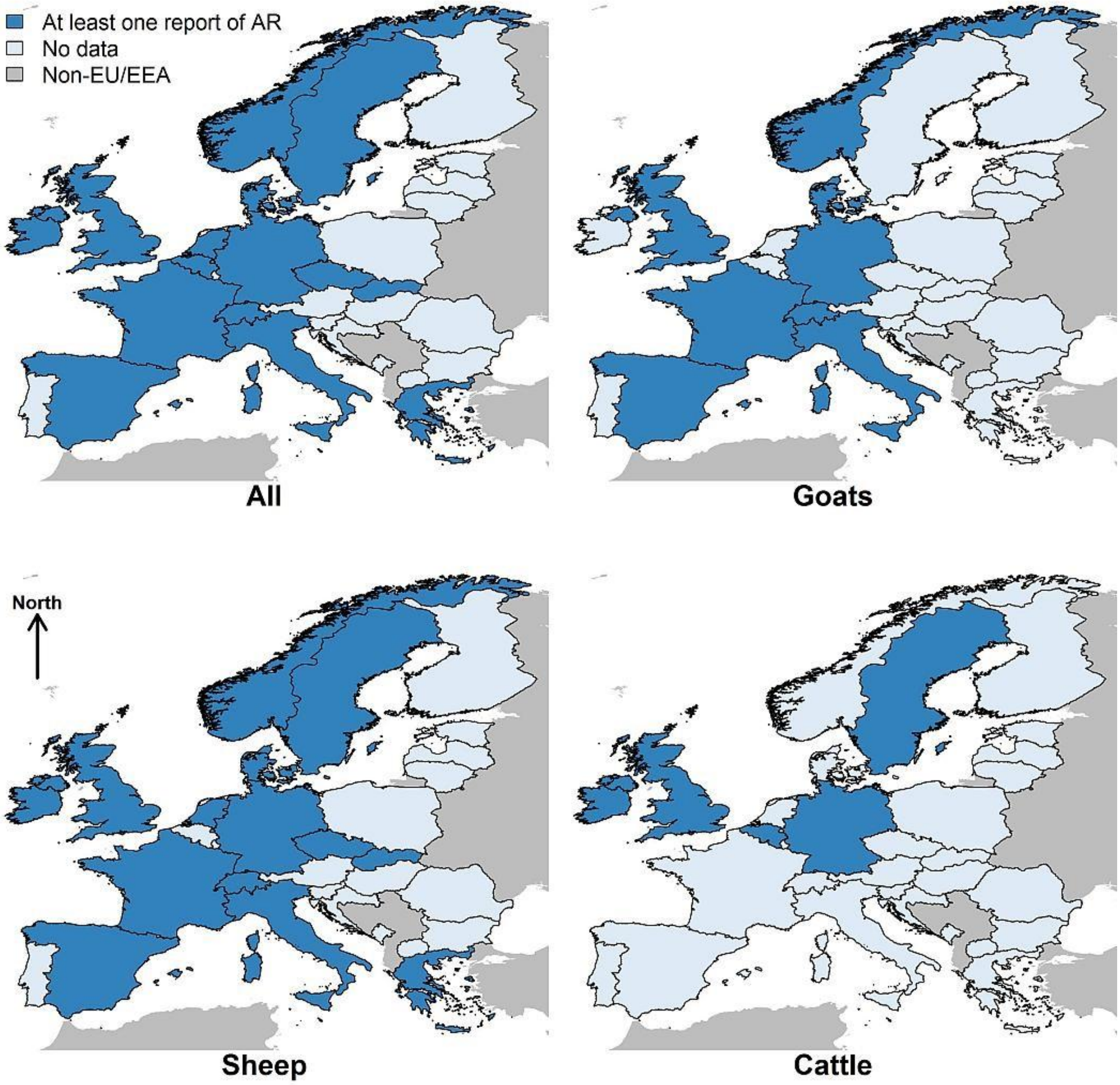
***Trichostrongylus* spp.**

BZ	**Sheep**	Denmark, France, Greece, Ireland, Italy, Netherlands, Norway, Slovakia, Spain, UK	Alvarez-Sánchez and others 2006, Bjørn and others 1991, Boersema and others 1987, Borgsteede and others 1997, 2007, Čerňanská and others 2006, Chartier and others 1998, Díez-Baños and others 2008, Domke and others 2012a, Geurden and others 2014, Good and others 2012, Maingi and others 1996b, Martínez-Valladares and others 2013, McMahon and others 2013, Mitchell and others 2010, Palcy and others 2010, Taylor and others 2009, Traversa and others 2007
	Goats	Denmark, France, Italy, Netherlands, Norway	Borgsteede and others 1996, Cabaret and others 1995, Chartier and others 1998, 2001, Cringoli and others 2007, Domke and others 2012a, Maingi and others 1996a, Paraud and others 2009, Zanzani and others 2014
ML	**Sheep**	Denmark, Greece, Italy, Netherlands, Slovakia, Spain, UK	Alvarez-Sánchez and others 2006, Bartley and others 2006, Borgsteede and others 1997, Čerňanská and others 2006, Geurden and others 2014, Maingi and others 1996b, Martínez-Valladares and others 2013, McMahon and others 2013, Traversa and others 2007
	Goats	Denmark, Italy, Switzerland	Artho and others 2007, Maingi and others 1996a, Murri and others 2014, Zanzani and others 2014
LEV	**Sheep**	Denmark, France, Greece, Ireland, Italy, Netherlands, Spain, UK	Alvarez-Sánchez and others 2006, Bjørn and others 1991, Borgsteede and others 1997, Chartier and others 1998, Geurden and others 2014, Good and others 2012, Maingi and others 1996b, Martínez-Valladares and others 2013, McMahon and others 2013, Mitchell and others 2010, Taylor and others 2009, Traversa and others 2007
	Goats	Denmark, France	Chartier and others 2001, Maingi and others 1996a, Paraud and others 2009

MDR	**Sheep**	Denmark, Germany, Greece, Ireland, Italy, Netherlands, Spain, UK	Alvarez-Sánchez and others 2006, Borgsteede and others 1997, Geurden and others 2014, Good and others 2012, Maingi and others 1996b, Martínez-Valladares and others 2013, Mitchell and others 2010, Traversa and others 2007, Voigt and others 2012
	Goats	Denmark, France	Chartier and others 2001, Maingi and others 1996a
Haemonchus contortus			
BZ	**Sheep**	France, Germany, Greece, Netherlands, Norway, Slovakia, Sweden, Switzerland, UK	Boersema and others 1987, Borgsteede and others 1997, 2007, Borgsteede and Duyn 1989, Cawthorne and Cheong 1984, Čerňanská and others 2006, Domke and others 2012a,b, Gallidis and others 2012, Geurden and others 2014, Grimshaw and others 1994, Hertzberg and others 2000, Höglund and others 2009, Hong and others 1992, Jordi 1980, Meyer 2001, Scheuerle and others 2009
	Goats	France, Netherlands, Switzerland	Borgsteede and others 1996, Cabaret and others 1995, Chartier and others 1998, 2001, Hertzberg and others 2000, Meyer 2001, Schnyder and others 2005
ML	**Sheep**	Czech Republic, Germany, Greece, Italy, Netherlands, Slovakia, Switzerland	Artho and others 2007, Borgsteede and others 1997, 2007, Čerňanská and others 2006, Geurden and others 2014, Scheuerle and others 2009, Vadlejch and others 2014, Zanzani and others 2014
	Goats	Germany, Switzerland	Artho and others 2007, Murri and others 2014, Scheuerle and others 2009, Schnyder and others 2005
LEV	**Sheep**	Greece, Netherlands	Borgsteede and others 1997, Geurden and others 2014
	Goats	France	Chartier and others 2001
MDR	**Sheep**	Greece, Netherlands	Borgsteede and others 1997, Geurden and others 2014, Van den Brom and others 2013
	Goats	France, Switzerland	Chartier and others 2001, Schnyder and others 2005
Ostertagia ostertagi			
BZ	**Cattle**	Belgium[b]	Borgsteede and others 1992

ML	**Cattle**	Germany, Sweden	Demeler and others 2009
Cooperia oncophora			
ML	**Cattle**	Belgium, Germany, Ireland, Sweden, UK	Areskog and others 2013, Bartley and others 2012, Coles and others 1998, Demeler and others 2009, El-Abdellati and others 2010a,b, McArthur and others 2011, O'Shaughnessy and others 2014, Stafford & Coles 1999
Other[c]			
BZ	**Sheep**	Ireland, Netherlands, Slovakia, Spain, UK	Bartley and others 2003, Borgsteede 1986, Burgess and others 2012, Calvete and others 2012, Grimshaw and others 1994, Keane and others 2014, Praslička and others 1994, Várady and others 2006, de Waal, T. and others unpublished observations
LEV	**Sheep**	Ireland, UK	Burgess and others 2012, Grimshaw and others 1994, Keane and others 2014
ML	**Sheep**	Ireland	Keane and others 2014
MDR	**Sheep**	UK	Burgess and others 2012

[a]AH = anthelmintic, BZ = benzimidazoles (including pro-BZs), ML = macrocyclic lactones, LEV = levamisole, MDR = multiple drug resistance
[b]Suspected resistance
[c]Other minor species, or species/genera not identified



Supplementary figure. The distribution of reported cases of anthelmintic resistance (AR) in the European Union, European Economic Area and Switzerland, at national level, based on the systematic review. Shaded countries had at least one reported case of AR. Note that regional distribution within countries is not plotted: see supplementary table for details of drug classes and GIN genera/species, and individual references for specific locations and apparent prevalence. No data were available for Iceland (not shown).